GROWTH AND GENETIC ANALYSIS OF PEJIBAYE

(*Bactris gasipaes* KUNTH, PALMAE) IN HAWAI'I.

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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Abstract

Pejibaye or peach palm (*Bactris gasipaes*, Kunth) was introduced into Hawai'i to supply the gourmet market with fresh heart of palm. New crop introduction requires evaluation of crop adaptation to its new environment and planning for future development, including genetic improvement. Leaf number of open-pollinated Benjamin Constant (Putumayo landrace) progenies was lower at harvest (6-8) than elsewhere (8-10), and offshoot number dropped dramatically from first harvest (6.5) to second harvest (2). Allometric equations for estimating whole plant leaf area and biomass were developed, using height and leaf number predictors. No significant plant population (density) effects on individual plant dimensions or growth were found over the range of 3333 to 6666 plants/ha. Relative growth rate (RGR) and unit leaf rate (E_A) between nursery and first harvest were highly correlated (r = -0.99 and -0.95, respectively) with earliness (days to harvest). The early progenies partitioned photoassimilates differently: two had high E_A, while one had moderate E_A and partitioned preferentially to leaf area, resulting in a higher leaf area ratio. Heart of palm yields were close to 900 kg/ha after 12 months of harvest and 1400 kg/ha after 18 months, both comparable to tropical American yields. When edible stem and leaf were added to the yields, these increased to 2.8 and 4.5 t/ha of marketable product, respectively. Quantitative genetic analysis of growth parameters suggested high levels of inbreeding in the germplasm studied, since the narrow-sense heritabilities were double those observed in other perennials. Additive genetic variances for RGR and earliness suggested the potential for significant response to selection, but phenotypic variation varied depending on the interval over which RGR

was estimated. The lowest estimate of RGR (over an entire development phase) provided the smallest response to selection but is similar to the response observed in other crops. Allozyme heterozygosity was remarkably low, ranging from 0.038 to 0.099, with a mean of 0.074, on par with inbred crops, rather than outbreeders. There was a lack of correlation between allozyme heterozygosity and growth parameters and morphological traits.

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List of Abbreviations

CGR Crop Growth Rate (kg ha^{-1} $year^{-1}$), where kg are dry weights

CV_P Coefficient of Phenotypic Variation [($V_P^{½}$ / mean) * 100]

E_A Unit Leaf Rate (g m^{-2} day^{-1}), also called Net Assimilation Rate, where g are dry weights

h^2 Narrow Sense Heritability (V_A / V_P)

LAI Leaf Area Index

LAR Leaf Area Ratio (m^2 kg^{-1}), where kg are dry weights

RGR Relative Growth Rate (g kg^{-1} day^{-1}), where g and kg are dry weights

V_A Additive Genetic Variance

V_P Phenotypic Variance

Preface

This dissertation is part of a larger project to introduce and evaluate pejibaye as a source of fresh hearts of palm in Hawai'i. In turn, this project is part of a larger effort to make the pejibaye a major world crop, returning it to the place of prominence that it held in tropical America at the time of the European conquest.

Although the pejibaye was important to the natives of tropical America as a fruit crop, and this remains its major long-term potential, its development as a source of hearts-of-palm is currently attracting major agroindustrial investment in Latin America. Because of the rather peculiar flavor and texture of its fruit, and the mild pleasant flavor and crispy texture of its fresh heart, the latter use was thought to be more attractive to consumers in the rich countries of the temperate zone.

Hawai'i is the only state in the United States that is capable of producing pejibaye hearts-of-palm economically in the field and is ideally situated culturally to introduce the fresh heart to the markets of North America and Asia. At the same time that it contributes to the development of the world market for pejibaye hearts-of-palm, Hawai'i can exploit pejibaye as a new crop for its diversified agriculture sector. Because its heart has a high unit value and because the species is well adapted to several of Hawai'i's agricultural zones, pejibaye hearts can certainly become an important niche market here. As it expands to fill this niche, the pejibaye will provide important economic returns to those who produce it and will stimulate Hawai'i's epicurean culture by enriching both oriental and occidental culinary traditions.

To further this effort, both the United States Department of Agriculture and the Governor's Agricultural Coordinating Committee have funded research necessary to introduce and evaluate the pejibaye as a plantation crop in Hawai'i. This larger effort introduced three populations of pejibaye from tropical America: the Benjamin Constant (Brazil) population of the Putumayo landrace, the subject of this dissertation; the Yurimaguas (Peru) population of the Pampa Hermosa landrace; and the San Carlos (Costa Rica) population of the Guatuso landrace. Both the Benjamin Constant and the Yurimaguas germplasms have proven to be attractive to consumers; at this writing, the San Carlos germplasm has not been used because of the high frequency of spiny plants in the seed lot received. Several farmers have already planted the first commercial acreage of pejibaye on the Big Island, on Oahu, and on Maui, and its short-term future appears promising.

Consequently, this dissertation and similar work with the Yurimaguas and San Carlos populations will help direct the improvement of pejibaye for hearts of palm in Hawai'i and thus contribute to the development of a new niche in Hawai'i's diversified agriculture sector. To further this development, the information collected by this project, part of which is presented here, is available to other researchers upon request. Please contact Dr. Richard M. Manshardt, Department of Horticulture, University of Hawai'i at Manoa.

Chapter 1. Introduction

During the last two decades, pejibaye or peach palm (*Bactris gasipaes*, Kunth) has been the subject of intensive research and development in various parts of tropical America (Mora Urpí 1992, Clement 1995). In 1990, it was introduced into Hawai'i with the intention of developing a niche market for its fresh heart-of-palm (Clement et al. 1993). The rapid introduction of any new crop requires an evaluation of the crop's adaptation to its new environment, both physical and commercial, as well as planning to assure its continued development in the event that the introduction attracts farmer and consumer interest. This study examines some of the biological and genetic aspects of this introduction program that are essential for evaluating adaptation and planning for future development.

Pejibaye is the Neotropic's only fully domesticated palm (Clement 1992). The Amerindians of tropical America domesticated it for its fruit, which was a starchy staple as important as manioc (*Manihot esculenta* Krantz) and maize (*Zea mays* L.) in some areas. During the last two decades, however, it has been most intensively planted for its heart-of-palm, a gourmet vegetable obtained from its crown (Mora Urpí et al. 1991, Tabora et al. 1993). The heart-of-palm is composed of the tender unexpanded leaves just above the apical meristem, wrapped within the tender petiole sheath of the spear leaf. World trade in canned heart-of-palm has been expanding at a rate of 10% per year since the mid-1980's, although there has been considerable year to year variation (Mora Urpí et al. 1991). To supply this growing demand there are at least 5000 hectares planted in

tropical America at this time, and the demand for seed in several countries suggests that field plantings will continue to expand rapidly.

The gourmet nature of the pejibaye heart-of-palm suggested the possibility of supplying the fresh product to chefs at up-scale restaurants. Most gourmet vegetables are worth more fresh than canned, and the premium for a fresh product, over a canned one, could allow a Hawaiian farmer to make a profit, even though production costs are much higher in Hawai'i than in almost any other part of the world. The pejibaye also supplies two other fresh vegetable products from the same shoot cut for heart: the edible stem, from just below the meristem, and the edible leaf, from just above the heart. Both of these products attracted chef and consumer attention in Hawai'i, although both are usually discarded in tropical America. Once a market potential was identified, the major questions became biological and genetic: does the crop grow well enough in Hawai'i, and is there good quality germplasm available for the farmer.

Pejibaye is a pinnate-leaved, suckering palm, frequently armed with spines on various surfaces. Good morphological descriptions of the mature pejibaye exist because of interest in the fruit, e.g., Clement (1995), but there are none of the juvenile plant. In palms, growth and development of the plant has been divided into several phases: the seedling, the establishment, the adult vegetative and the adult reproductive phases (Tomlinson 1990). For heart-of-palm production, only the first three of these phases are of interest, since the shoot will be harvested at the end of the establishment phase or beginning of the adult vegetative phase. In this study, the morphology of each vegetative phase was described, giving special attention to traits that influence growth, long-term

productivity of a plantation, and ease of management, i.e., leaf and offshoot number, and the presence of spines, respectively.

Before proceeding with an evaluation of pejibaye growth in Hawai'i, it was necessary to develop a set of equations to estimate whole plant leaf area and biomass non-destructively. The non-destructive criterion was necessary because some of the same plants being evaluated might be required in a future improvement program. Most previous work on estimation equations for pejibaye had followed the African oil palm (*Elaeis guineensis* Jacq.) model of estimating individual leaf area and biomass, counting leaf number, adding stem biomass, and obtaining estimates of whole plant leaf area and biomass (Corley & Gray 1976). Szott et al. (1993), however, followed the dicot tree model, which involves direct estimation of whole plant leaf area and biomass from a small set of plant dimensions (Crow & Schlaegel 1988). Both of these approaches were examined in this study, and the latter was selected for general use because of the large number of individual plants to be measured.

Classical growth analysis methodology (Radford 1967) was used to evaluate growth and adaptation to three Hawaiian environments chosen to represent areas where new agricultural options are required (the Hamakua coast and south Hilo on the Island of Hawai'i, and the north shore of the Island of Oahu). With this methodology, growth over various intervals was examined, including uniform time intervals, uniform developmental phases, and mixed intervals, as suggested by Coleman et al. (1994). Uniform time intervals allow for the examination of growth under similar climatic conditions, but have the disadvantage of grouping plants that are in different phases of development. Uniform

developmental phases, on the other hand, group similar plants, but have the disadvantage of including variable amounts of climatic variation because of varying plant growth rates. A high correlation between growth rate over different intervals with earliness, measured as days to harvest, can help identify outstanding individual plants and progenies that are well adapted to the environment and merit selection for use in a breeding program.

Planting density affects the growth of individual plants, the yield from a given area, and the return that the farmer can expect from the plantation. Preliminary research on planting density for pejibaye heart-of-palm was reviewed by Clement (1993). Increases in density generally decreased individual heart weight and offshoot number. Although heart weight per plant decreased, total yield increased over the densities examined. Three densities were examined in this trial: 5000 plants/ha, the commercial density in Costa Rica and most other countries, and 3333 and 6666 plants/ha.

Yield is of primary interest to the farmer and plant breeder. In the case of pejibaye for heart-of-palm, yield is a small fraction of whole plant biomass at the end of the establishment phase. Yield is an extremely complex trait that usually responds slowly to selection pressure (Simmonds 1979). Because plant size is the criterion for harvest, yield depends more on earliness (days to harvest) than on weight of the individual heart. Classical growth analysis provides several measures of growth which are correlated to earliness as defined here. The most basic parameters, e.g., relative growth rate, unit leaf rate, and leaf area ratio, have been targeted for selection in various crop improvement programs, with mixed success (Gupta 1992).

Success in a breeding program depends upon the amount of phenotypic variation present in the base population, the proportion of phenotypic variation determined by additive genetic effects (from which the narrow sense heritability is estimated), and the selection intensity practiced (Simmonds 1979). The first two criteria mentioned are basic to the field of quantitative genetics. The experimental designs used in the current study were chosen to allow estimates of the phenotypic and additive genetic variances, and the narrow sense heritability of each trait of interest. The precision of estimation of these variables was limited by of the number of individuals in each progeny, the size of each experiment, and the lack of knowledge about pejibaye's response to environmental variables. Nonetheless, they provide a useful guide for the breeder planning to improve pejibaye for heart-of-palm in Hawai'i.

Another estimate of genetic variability is provided by the variation in isoenzymes (Torres 1989). Preliminary work with isoenzymes in pejibaye had identified several enzymes with good activity and resolution after extraction from leaf tissue (Rojas Vargas 1993). In the current study, further work on methodology permitted the visualization of numerous enzymes, the probable genetic interpretation of several of them, and a population genetic analysis of allozyme variation. In most naturally out crossing crops, high yields are generally the result of heterosis (Simmonds 1979). Consequently, correlations between allozyme heterozygosity and growth or yield may exist (Stuber 1991). The current study permitted the examination of these correlations in pejibaye, the first time that they have been examined in a palm.

The sequence of biological and genetic research followed in this study was designed to plan for an improvement program for pejibaye heart-of-palm production in Hawai'i. The results of this study can guide such an improvement program if pejibaye becomes an important enough crop to merit the investment. That decision will be made by the farmers and consumers of Hawai'i. If the farmers decide positively, baseline information is provided by this study to guide future work.

Chapter 2. Literature Review

2.1. Cytotaxonomic Background

Bactris gasipaes (Kunth) has 2n = 2x = 28 chromosomes (Mora Urpí 1984). Although *Bactris* is poorly studied cytologically, all other reports are of x = 14, also (Uhl & Dransfield 1987).

Bactris (Jacq. ex Scop.) contains more than 250 species, distributed in three subgenera. One subgenus has three sections and one section has an additional subsection (Sanders 1991). All are restricted to the Neotropics. The pejibaye is included within the subgenus *Guilielma*, which contains 3-8 species, depending upon the taxonomist. A. Henderson (New York Botanical Garden) and J.-J. de Granville (ORSTOM) have initiated a much needed systematic study of *Bactris* that will certainly reduce the number of species. Because *Guilielma*'s taxonomy is in flux, I will use Harlan & de Wet's (1971) gene pool concept to discuss phylogenetic relations.

The subgenus *Guilielma* is the pejibaye's secondary gene pool (GP-2). It contains a maximum of: *B. caribea, B. ciliata, B. dahlgreniana, B. insignis, B. jamaicana, B. macana*, and *B. setulosa*. Two of the species were added recently: *B. jamaicana* (Sanders 1991), and *B. setulosa* (Bernal & Henderson 1995). Several species have recently been challenged taxonomically and proposed as synonyms of *B. gasipaes*: *B. ciliata* (Bernal 1989), *B. insignis* (Balslev & Moraes R 1989), and *B. dahlgreniana* (Bernal & Henderson 1995). Bernal & Henderson (1995) also propose that *B. caribea* is a synonym of *B. macana*.

All undomesticated species are small fruited (1-10, rarely 20 g) with large numbers of fruit/bunch (400-1500), but vegetatively similar to pejibaye (especially those in southwestern Amazonia). They occur allopatrically in northwestern South America, except for *B. jamaicana* in the Caribbean. These species, and the spontaneous populations of pejibaye, occur principally in disturbed ecosystems, along river edges and in forest gaps. They require full sun to fruit; in its absence they may survive in the forest but do not reproduce. Local populations of pejibaye may form interspecific hybrids with members of this gene pool (GP-2) (Mora Urpí & Clement 1988).

The tertiary gene pool (GP-3) includes all other members of the genus. Species of the subgenus *Bactris sensu stricto* may also hybridize naturally with pejibaye.

The origin of pejibaye is still debated. Mora Urpí (1992) and Bernal & Henderson (1995) argue for a polyphyletic origin, with several local domestications at various places in the GP-2 range, especially in southwestern Amazonia and the Cauca and Magdalena River valleys in Colombia. A recent review of the genetic evidence for crop origins, however, suggests that polyphyletic origins are rare (Blumler 1992). How likely pejibaye is to be one of the rare cases is currently unknown.

Clement (1988, 1992) argued that a monophyletic origin is more likely and that the observed variation originated through Amerindian selection, germplasm migration, adaptation to a wide range of environments, and introgression with GP-2 and GP-3 species. In this case, the species was probably domesticated in southwestern Amazonia, where the most similar *Guilielmas* occur, principally *B. ciliata*, *B. dahlgreniana*, and *B. insignis*, one of which may be the progenitor. The fact that all three of these species have

recently been reduced to synonyms of *B. gasipaes* (Balslev & Moraes R 1989, Bernal 1989, Bernal & Henderson 1995) appears to support the monophyletic origin hypothesis, as these would then become the most primitive pejibayes based on fruit morphology. Bernal & Henderson (1995) argued for the opposite conclusion, however.

The reasons for the original domestication are also debated, as the smallest fruited *Guilielmas* appear to offer little attraction to a hungry hunter-gatherer (Clement et al. 1989). V.M. Patiño (personal communication, 1990) suggested that the wood was the first attraction. In fact, among modern Amerindians it is a preferred wood for the manufacture of numerous subsistence artifacts, as well as being used for construction. A second possibility is the mesocarp oil, which in *B. dahlgreniana*, for example, averages 60% of dry weight (Clement et al. 1989). After the original domestication, starch became an important factor and appears to have become the dominant factor in the 'mesocarpa' and 'macrocarpa' landraces (Mora Urpí 1984).

From southwestern Amazonia, the pejibaye was distributed by the Amerindians throughout the region and progressively modified by their selection pressures. Well after domestication had started (perhaps as early as 10,000+ BP?), the species was taken to the Pacific coast of Colombia and Central America (Clement 1986b). Mora Urpí (1992) cites archeological remains at 4000 BP in Costa Rica. This northwestward migration resulted in significant vegetative differences (many more spines, larger leaves, stouter stems in the Occidental complex), due to introgression with local, possibly GP-3 species. Reports of *Guilielma*-like, undescribed populations in Darién (Panamá) and the Pacific coast of Ecuador (Mora Urpí 1992) may be GP-2 species or migration relicts.

Today there is a complex landrace pattern (Mora Urpí 1984, 1992, Clement 1988, Mora Urpí & Clement 1988). This complex has been divided into Occidental and Oriental subcomplexes based upon vegetative differences (Mora Urpí 1984), and further into classes based upon fruit size (Mora Urpí & Clement 1988, Mora Urpí 1992): the Pará, Juruá and Rama 'microcarpa' landraces have fruit weighing 10-30 g; the Pampa Hermosa, Tigre, Pastaza, Solimões, Inirida, Cauca, Darién, Utilis and Guatuso 'mesocarpa' landraces have fruit weighing 30-70 g; the Putumayo and Vaupés 'macrocarpa' landraces have fruit weighing 70-250 g. Fruit size reflects the degree of modification that occurred during pejibaye's domestication, although other traits were also modified significantly (Clement 1992).

The less modified 'microcarpa' landraces have more mesocarp oil, fiber and carotene, smaller mesocarp percentages (70-85%), smaller harvest indices, and more spines on the stem and leaves. The 'mesocarpa' landraces are intermediate in most of these traits, although some have high frequencies of spinelessness (e.g., Pampa Hermosa, Guatuso), while others are exceptionally spiny (e.g., Pastaza, Cauca, Utilis). The 'macrocarpa' landraces have extremely starchy, low fiber and carotene mesocarps, very high mesocarp percentages (> 95%), high harvest indices, and fewer spines (Clement 1992).

The primary gene pool of pejibaye is composed of the above described landrace complex, designated the *utilis* subspecies (Clement 1995). There are also several spontaneous populations that may represent very early domesticates or truly wild populations (especially if some GP-2 species are reduced to synonymy with *B. gasipaes*). This group of populations is designated the *speciosa* subspecies (Clement 1995).

Clement & Mora Urpí (1987) suggested that different landraces have specific characteristics that can be exploited for different objectives within each national breeding program. Clement et al. (1988) identified three populations, each in a different landrace, that could serve as the genetic base for a heart-of-palm improvement program: Benjamin Constant, Brazil (Putumayo landrace); Yurimaguas, Peru (Pampa Hermosa landrace); and San Carlos, Costa Rica (Guatuso landrace). The major criterion was spinelessness, but rapid growth, as reflected in other morphological characteristics, is essential (Table 2.1).

Table 2.1. *In situ* population means of several important traits for improving pejibaye for heart-of-palm production (Clement et al. 1988).

Population	Stem Diameter (cm)	Internode Length[a] (cm)	Number Leaves	Leaf Rachis Length (m)	Leaf Area (m^2)
B. Constant	19.0	20.3	17.2	2.88	3.9
San Carlos	17.8	13.3	16.4	2.98	4.3
Yurimaguas	18.0	19.2	14.6	3.18	3.3

[a] Mean of first nine internodes

2.2. Morphology of the Mature Pejibaye

The pejibaye is extremely variable morphologically, both locally and across its range, especially for fruit morphology and composition and spine characters. It is also well adapted to a wide variety of environments, fruiting in locations from 0 to 900+ m above sea level, with 1500 to 8000 mm of rainfall and dry seasons of less than six months duration. The pejibaye grows on soils ranging from nutrient-poor Oxisols and Ultisols to nutrient-rich Alfisols, but it requires full sun and good drainage.

The pejibaye is a caespitose palm. The stem internodes are generally heavily armed with thin, strong black spines of different sizes, although some populations have been selected by the Amerindians for spinelessness (Mora Urpi et al. 1984). Stem diameter varies from 10 to 30 cm. Stem internode length varies from 5 to 40 cm during the early years (Clement 1986b), after which it becomes progressively reduced to about 1 to 2 cm in older plants, as these change from a purely vegetative to a fully reproductive phase. The plant may attain heights of 15 to 20 m quite rapidly, which is a major problem for fruit harvesting.

The leaves are pinnate, generally with a spiny petiole and rachis, and frequently with spiny leaflet veins and edges. The mature leaf petiole ranges from 100 to 200 cm in length, while the mature leaf rachis ranges from 100 to 300 cm, and the whole frond curves downward with age. The reduplicate leaflets number from 100 to 300 and are arranged in groups of 2 to 8 along the rachis, with each leaflet inserted at a different angle within the group, giving the frond a "shaggy" appearance. Leaflets range from 50 to 120 cm in length and 20 to 60 mm in width. Individual leaf area ranges from 2 to 6 m^2 and leaf biomass from 0.7 to 1.2 kg (Clement & Mora Urpí 1983, Clement et al. 1985, 1990, Clement 1986b, Gutierrez M et al. 1986).

The inflorescences are monoecious, arising in the axil of each leaf and becoming visible as the leaf enters senescence or has already died. The inflorescence peduncle varies from 30 to 60 cm in length, and the rachis from 20 to 50 cm, with between 20 and 80 flower-bearing rachillae (Clement 1986b). Each rachilla has several to more than two dozen pistillate flowers and several hundred to more than a thousand staminate flowers.

The inflorescence may contain anywhere from 25 to 1,200 pistillate and 10,000 to 30,000 staminate flowers (Valle Bourrouet 1986). Although there is the potential for one inflorescence in each axil, this is rarely realized, since drought, poor plant nutrition, excessive yield in the previous year and other, as yet undetermined factors can cause abortion (Clement 1987).

Mora Urpí & Solis (1980) determined that pejibaye is principally allogamous, due to the protogynous maturation of the pistillate and staminate flowers. Pollination is entomophylous, by various species of *Derelomus* and *Phylotrox*, both Curculionidae genera. Wind or gravity pollination can also occur. There may be a genetic mechanism to reenforce the probability of cross fertilization (i.e., partial or complete self-incompatibility) (Mora Urpí 1980). Clement & Arkcoll (1984), working with two newly mature plantings of the Benjamin Constant population (Putumayo landrace), found 19 and 37% mean self-fertility, with variation between individuals ranging from 0 to 82% self-fertility. The sample with 37% self-fertility produced the seed used in this project. Thus, pejibaye is principally allogamous, but varying degrees of autogamy can be expected in any population, given that bunches on different stems of the same clump may flower at the same time and that the self-sterility mechanism is only partial.

2.3. Phases of Growth in Palms

The concept of distinct growth phases in palms is widely used for demographic studies (Tomlinson 1990), as well as general morphology of economic palms, e.g., Hartley (1977) and Child (1974). Tomlinson (1990) defines five growth stages:

embryonic, seedling, establishment, adult vegetative, and reproductive (Figure 2.1). The embryonic phase extends from fertilization of the ovary to germination of the embryo, where germination is defined as the end of embryo dormancy. The seedling phase extends from germination to the depletion of endosperm energy and nutrient reserves. Since depletion of endosperm reserves can not be observed morphologically, the end of this phase is somewhat arbitrary. The establishment phase extends from depletion of endosperm reserves to the maximum expansion of the stem diameter. During this phase, leaf morphology changes from the early juvenile eophylls to mature pinnate leaves. In branched palms, like pejibaye, this is the phase in which branch primordia are formed and start to expand. The end of this phase is clearly visible in most palms when the stem starts to elongate at ground level. The adult vegetative phase extends from the beginning of stem elongation to the appearance of the first inflorescence. The reproductive phase completes the palm's life cycle, during which most palms produce reproductive organs regularly.

In the current work, the seedling and establishment phases are of most interest. Tomlinson (1990) emphasizes that there is considerable within-species variation for growth rates during all phases. There may be correlations between growth rates in one phase and growth rates in another, as occurs in coconut (*Cocos mucifera* L.) (Rognon 1972a, b, Ratna Kumar et al. 1993). If these exist, they may allow for very early selection of outstanding individuals and progenies with respect to growth rates.

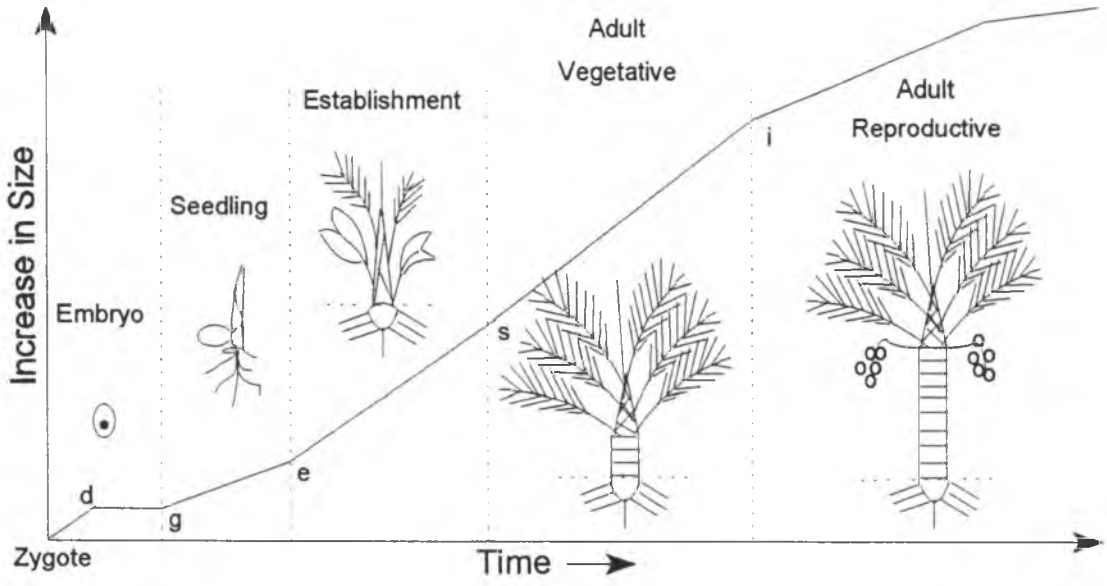

Figure 2.1. The phases of development in palms (after Tomlinson 1990). Point 'd' refers to embryo dormancy, 'g' to germination, 'e' to exhaustion of seed reserves, 's' to stem achieves maximum diameter and appears at ground level, and 'i' to inflorescence appearance.

In pejibaye grown for its heart, the establishment phase essentially extends from field planting to harvest. In Costa Rica, palms are harvested for their hearts before the stem starts to elongate (Mora Urpí, personal communication). In the current study, harvest occurs soon after stem elongation has started, so that a larger edible stem biomass can be obtained.

2.4. Germination of Pejibaye

All palms have hypogeal germination, where the cotyledon remains within the endocarp (Tomlinson 1990). Although previous students of pejibaye germination have not classified its type of germination, I conclude that pejibaye germination corresponds to

the 'adjacent ligular germination' type, similar to that of African oil palm (*Elaeis guineensis* Jacq.) (Tomlinson 1990).

Recent work on pejibaye seed germination has shown that pejibaye has a recalcitrant seed (Ferreira & Santos 1992), which loses viability rapidly when dried. Viability and vigor of emergence are strongly affected by the rate of drying also (Ferreira & Santos 1993).

While Ferreira & Santos (1992, 1993) regularly use sawdust as a germination substrate, both Villalobos & Herrera (1991) and Clement & Dudley (1995) found that sand of various types is equally satisfactory. Use of bottom heat to accelerate germination does not give significantly better results than no heat, in the temperature range of 30 to 35°C (Villalobos & Herrera 1991, Clement & Dudley 1995), while 40°C is lethal (Villalobos & Herrera 1991).

In coconut, precocious and vigorous germination are used as selection criteria for planting materials because they are correlated with later growth and precocious flowering (Liyanage 1967, Rognon 1972a, b, Ratna Kumar et al. 1993). Since production of palm heart depends essentially on vigorous juvenile growth, Clement et al. (1988) suggested that there may also be a correlation between germination parameters and later growth that is worth pursuing in pejibaye. Ferreira & Santos (1992, 1993) use an emergence velocity index (EVI) (Popinings 1977) or speed of germination index (Copeland & McDonald 1985), which is essentially a measure of the rate of germination for a given progeny, where:

$$EVI = \sum [(\text{number of plants emerged on day } x)/x],$$

and where x is days after germination starts. The calculations start on the day that the first seed germinates and stop when no more seeds germinate. A rapidly germinating progeny will have a high index and a slow progeny will have a low index. This index is positively correlated with later vigor (Copeland & McDonald 1985).

2.5. Allometric Relationships in Plants

Allometry is the study of the relationships among the dimensions of an organism. In all organisms, growth in one part is generally related to growth in another part or of the whole. For example, growth in height of a dicot tree is accompanied by growth in stem diameter, so that mechanical stability is maintained. The relationships among parts and of parts with the whole can be used to estimate whole plant dimensions as well (Causton & Venus 1981).

There are two ways of conceptualizing a palm such as coconut, African oil palm, or a single stem of pejibaye: 1) as a collection of organs that can be studied individually and put together into a unit; or 2) as an integrated whole that is studied as a unit. Both are valid and provide insights into the biology of the plant. The second concept is based upon the 'pipe model' theory of tree form, devised by Japanese forest ecologists (Shinozaki et al. 1964), to explain the mechanical and hydraulic constraints imposed on woody, self-supporting plants as they grow in height (Tomlinson 1990). Tomlinson's (1990) discussion of the pipe model is summarized because he contrasts monocots and dicots.

In the pipe model, the 'unit pipe' is considered as an abstract segment of the axis supporting a 'unit of crown' mechanically and supplying it hydraulically. Thus, an

individual tree is an aggregate of pipes and their associated crown units. The model has been used by ecologists to establish quantitative relationships (allometric relationships) between trunk and crown biomass. There are clear contrasts between dicotyledons and monocotyledons that are important.

In dicots, the trunk and the crown maintain constant proportions, although the relationship is not necessarily linear. As the number of crown units increases, so does the number of pipes, which is not fixed. There is a progressive replacement of both types of unit. As older crown units (e.g. leaves or branches) are lost, the supporting pipes become useless hydraulically but continue to function mechanically in the support of the trunk and crown. Successive crown units develop and are supported at progressively greater heights. The replacement of old pipes by new ones comes about by secondary growth, i.e., an increase in trunk diameter. In these trees, the pipe is a definite abstraction, because the growth increments of new vascular tissue are laid down in the form of a hollow cylinder of vascular tissue. Essentially, the cylinder of vascular tissue is the pipe, but individual vessels only function as true pipes for a limited time.

In monocots, the trunk and crown do not maintain a constant proportion. The crown develops to its mature size during establishment growth and is connected to the root system by an appropriate number of pipe units, each of which is short because a tall trunk has not yet developed. Growth in height subsequently involves the extension of a fixed number of pipes, since there is no replacement of pipes. As crown units (which are single leaves) fall, only the short and immediate connection to the permanent pipe ceases to function hydraulically. The unit pipe effectively grows in length and functions

throughout the life span of the tree, both in conduction and support. The unit pipe, therefore, is less of an abstraction in a palm because the fibrovascular bundles are discrete units.

In dicots, trunk height and diameter (at a fixed height, usually 1.3 m) are potential predictors of tree and crown biomass and leaf area. They may be used individually or in combination, e.g., Crow & Schlaegel (1988), and Waring (1983). Szott et al. (1993) found that the same holds true for pejibaye.

Just as trunk height and diameter can be used to predict crown biomass, petiole cross-sections or rachis length can be used to predict leaf biomass, e.g., Corley et al. (1971) and Clement et al. (1990). Theoretically, individual leaf area can be predicted from petiole cross-section also, but another method has been used by researchers working with African oil palm and pejibaye.

2.5.1. Allometric Relationships in African Oil Palm

J. J. Hardon, R. H. V. Corley and their colleagues pioneered the study of the allometric relationships among plant dimensions in the African oil palm to find non-destructive methods for estimating plant dimensions and growth. The oil palm researchers consider the palm to be a simple multi-component system, with a trunk from which leaves and inflorescences develop sequentially (Corley et al. 1971). Prediction equations were developed for each plant component and the components are added to obtain a whole.

Hardon et al. (1969) estimated individual leaf area from the rectangular leaf area by the equation:

$$L_A = b(n \times lw)$$

where L_A is leaf area in m^2, n = number of leaflets, lw = mean length x mid-width (m) of a sample of the largest leaflets chosen from the middle of the leaf rachis, and b = 0.55 (r^2 = 0.99). They also found that measuring two leaves per plant was sufficient to obtain a good estimate of mean leaf area in the crown of an adult reproductive phase plant.

Corley et al. (1971) estimated individual leaf biomass from leaf petiole dimensions by the equation:

$$L_W = 0.206 + 0.102P$$

where L_W is leaf biomass in kg and P = petiole width x depth (cm^2) at the insertion of the first leaflet (r^2 = 0.935). Measuring two leaves per plant was sufficient to obtain a good estimate of mean leaf biomass in the crown.

Corley et al. (1971) also estimated trunk biomass from the trunk volume and its density by the equation:

$$T = VD$$

where T is trunk biomass in kg, V is trunk volume ($\pi (d/2)^2 h$) and D is density, which varies with age and is predicted by the equation:

$$D = 83 + 7.62A$$

where D is density in g/l and A is palm age in years from transplanting. While there is an increase in trunk density from the apex to the base and the shape of the bole is not cylindrical, this equation was accurate (Corley et al. 1971).

Corley & Hardon (unpublished, cited by Corley (1976)) estimated the area of the bifid eophyll by the equation:

$$L_A = 0.5(l \times w)$$

where l is the length of one lobe and w is the maximum width of the bifid eophyll.

2.5.2. Allometric Relationships in Pejibaye

Because photosynthesis occurs in the leaf, it's morphology and allometry have received attention. Clement & Mora Urpí (1983) presented a detailed description of the pejibaye leaf and the variation observed in leaf dimensions. The leaflets are arranged in groups along the rachis, with the first leaflet of each group being nearly perpendicular to the rachis and each subsequent leaflet more horizontal. This arrangement gives the pejibaye leaf its characteristic "shaggy" appearance. It may also enhance light absorption efficiency, because it makes the leaf generally more "vertical." Leaflet length varies irregularly along the rachis, which makes standardized leaflet selection for measurement and area estimation more difficult.

Clement et al. (1985), working on Occidental complex germplasm, adapted the methodology of Hardon et al. (1969) for use with pejibaye. The principal modification was with respect to leaflet selection. On a mature plant leaf, the leaflets and groups of leaflets are counted and the first leaflets of the following groups are selected: 6th, 10th and 14th on the right side; 8th, 12th and 16th on the left side of the rachis. Leaflet length and maximum width are measured and averaged over the leaflets. Leaf area is then estimated by the following equation:

$$L_A = 0.583((\Sigma(l \times mw)/6) \times n)$$

where L_A is leaf area in m^2, l is leaflet length (m), mw is leaflet maximum width (m), 6 is the leaflet sample, and n is total leaflet number. The constant (0.583) adjusts the rectangular shape (l x mw x n) to the leaf's shape. This equation estimates leaf area with a relative absolute error of less than 5%. Martel & Clement (1986) validated this methodology on Putumayo, Solimões and Pará landrace populations and found that the slopes and intercepts for the different population equations were not significantly different, proving that this methodology can be used for the species as a whole. Clement (1986a) suggested that counting only the leaflets on the side of the rachis with the first leaflet and doubling this figure would reduce labor requirements somewhat without unduly raising the error expected.

Clement et al. (1990) found that the logarithmic transformation (base 10) of the petiole cross-sectional area allowed for the direct estimation of leaf biomass in three Amazonian populations (Pará, Pampa Hermosa, Putumayo landraces). Following Corley et al. (1971) they measured rachis width and maximum thickness at the point of insertion of the first leaflet (Figure 2.2). The transformed product of these measurements was equal to the transformed leaf biomass, and is estimated by the equation:

$$\text{Log}_{10} L_W = 0.97 \text{ Log}_{10} (w \times mt)$$

where L_W is leaf biomass in grams, w is leaf rachis width (mm) at insertion of first leaflet, mt is leaf rachis maximum thickness (mm) at the same point and 0.97 adjusts the estimate to leaf biomass in grams. When reverse transformed, this function estimates leaf biomass with approximately 12% relative absolute error. The slopes and intercepts of the

equations determined for the three populations were not significantly different, so the single equation serves for all Amazonian populations. This methodology has not yet been validated on the Occidental complex, which are somewhat different in leaf morphology and dimensions as compared to the Amazonian landraces, but it is expected to work well because the leaf area estimator does.

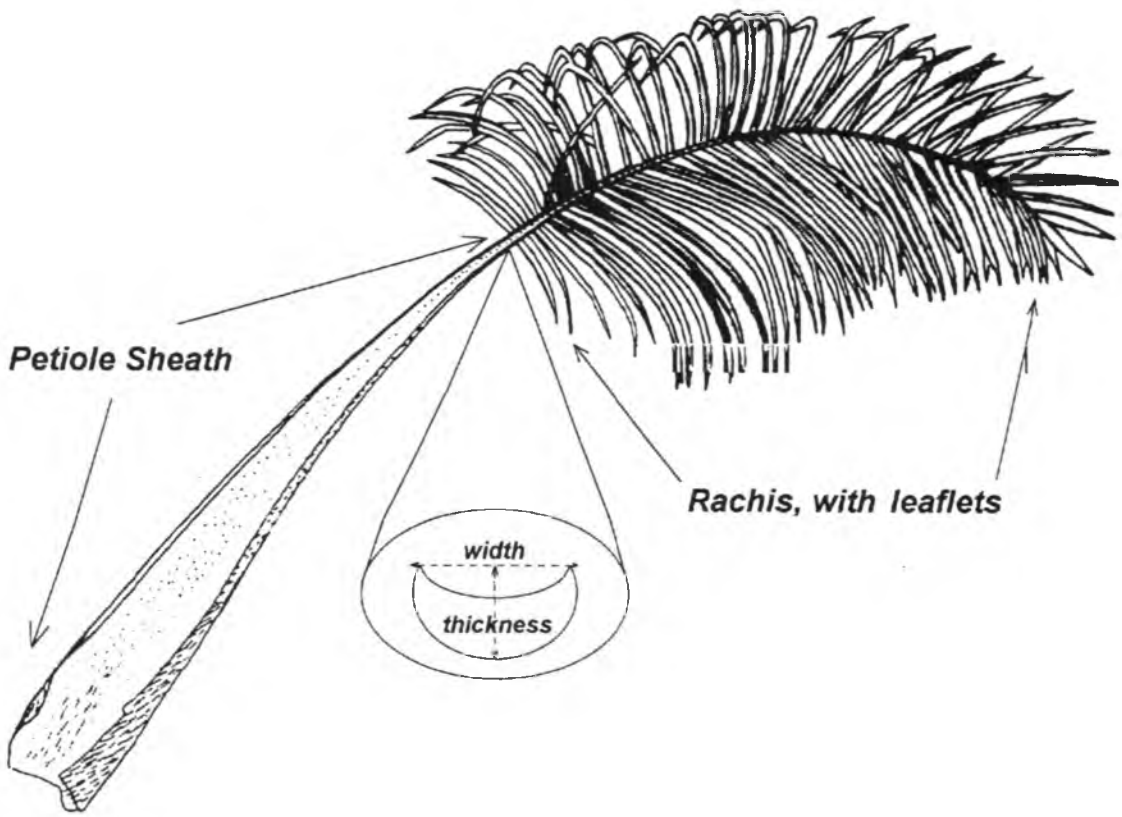

Figure 2.2. The mature pejibaye leaf. Just distal of the insertion of the first leaflet is the point at which the petiole/rachis width and thickness are measured. The petiole sheath can be divided into the sheath, with fibers along its edge, and the petiole, without fibers.

Clement & Habte (1994) estimated the area of the bifid eophyll leaf by the equation:

$$L_A = 1.107*(l \times w)$$

where L_A is leaf area in cm^2, l is the length of the leaf (cm) and w is half of the width of the leaf (cm), measured as shown in Figure 2.3 (the r^2 of 0.97 is only approximate because of the lack of an intercept in the regression equation). This estimator is very similar to that developed by Corley & Hardon (unpublished, cited by Corley (1976)).

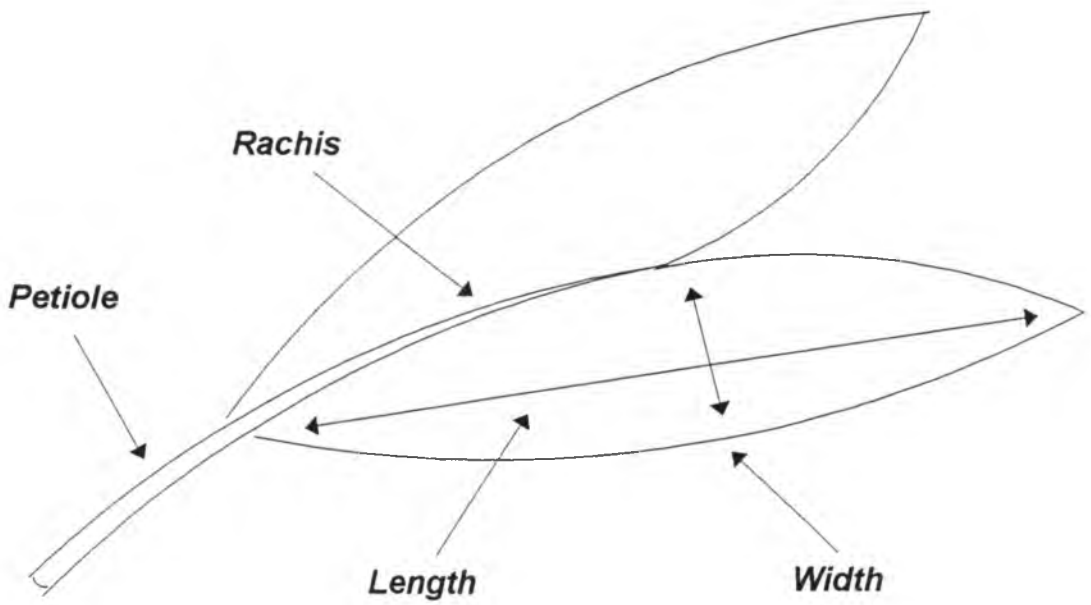

Figure 2.3. Schematic drawing of a pejibaye bifid eophyll, showing the dimensions measured in this study.

Szott et al. (1993) took the whole-plant allometric approach to estimate whole plant biomass, rather than the component approach used in African oil palm and by Clement and colleagues. They obtained allometric relationships for the Yurimaguas hybrid population (a hybrid between the 'mesocarpa' Pampa Hermosa and the 'macrocarpa' Putumayo landraces (Mora Urpí & Clement 1988)) for both adult vegetative and reproductive phases. They measured palm height (m) to the fork between the spear (or flag) leaf and the first fully expanded leaf in the crown. By convention, the spear leaf is

counted as leaf 0, the first fully expanded leaf as leaf 1, the second fully expanded leaf as leaf 2, etc, down to the oldest living leaf (Corley & Gray 1976a). They also counted total leaf number, stem diameter at 0.65 m (versus the more common 1.3 m or the base of the stem), and other dimensions that turned out to be less useful.

Szott et al. (1993) found two similar allometrics for the prediction of total above ground biomass for plants of any size. The equations were:

$$W = 0.735H^2,$$

and

$$W = 0.549H^2 + 0.077L^2,$$

where W is plant biomass (kg), H is plant height (m), and L is leaf number ($r^2 = 0.95$ and 0.96, respectively). Both of these allometrics overestimated plant biomass for juvenile plants, however. Consequently, they used only the smaller plants (<4.2 m, measured to the arc of the first fully expanded leaf) to develop another set of allometrics for the prediction of juvenile plant biomass. These equations were:

$$W = 0.032D^2,$$

and

$$W = -0.24 + 0.024L^2 + 0.020D^2,$$

where W is plant biomass (kg), L is leaf number and D is stem (occasionally crown shaft) diameter at 0.65 m ($r^2 = 0.89$ and 0.95, respectively). With a height of < 4.2 m, these plants were roughly equivalent to Tomlinson's adult vegetative phase (1990).

Szott et al. (1993) developed allometrics to estimate total trunk biomass, total leaf biomass, total leaflet biomass, and total petiole/ rachis biomass. The total leaf biomass allometrics for smaller plants are interesting. The equations were:

$$W_L = 0.129L,$$

and

$$W_L = 0.116D,$$

where W_L is total plant leaf biomass (kg), L is leaf number and D is stem diameter at 0.65 m ($r^2 = 0.93$ and 0.89, respectively). In most tree dicots, total plant biomass and total plant leaf area are also related to H and D (Crow & Schlaegel 1988)

2.6. Growth Analysis

Growth analysis is a methodology that assists in explaining plant growth, and finally yield, from the viewpoint of dry matter production. This is done by partitioning growth into a series of 'components of growth' (Causton & Venus 1981). The dry weight increments of plants over time give an indication of the overall growth of the plant. A measure to determine the plant's 'productive investment' is also required and this is provided by leaf area. The leaves are the most important photosynthetic organs, and light interception and photosynthesis depend ultimately upon the available leaf area, if the environment is favorable. In the growth analysis of pejibaye for the production of palm heart, the leaves are of major importance, since they account for most above ground biomass, even at harvest.

Growth analysis can play an important role in the comparison of genotypes of a species, often as part of a breeding program (Causton & Venus 1981). Growth analysis can also help explain how a given genotype responds to different environments, both biotic (inter- and intra-specific competition) and abiotic (soil, climate, etc.) (ibid.). Corley (1983) suggests that the improvement of the majority of tropical perennial crops could benefit substantially from the increased use of growth analysis in improvement programs.

In growth analysis, primary data (e.g., length, width, weight of leaves, stems, and fruit) are combined to derive several parameters that have physiological meaning at the whole plant level. These include:

Absolute growth rate. This is the change in weight over time and is expressed as:

$$GR = dW/dt,$$

where W is the total dry weight of the plant at time t. In crop growth analysis, this is generally expressed in metric tons/hectare/year, in which case it is the Crop Growth Rate (Radford 1967). The mean CGR over a time interval is calculated by the equation:

$$CGR = (W_2-W_1)/(t_2-t_1).$$

The only assumption involved in the use of this parameter is that W varies without discontinuity throughout the period t_1 - t_2 (Radford 1967).

Since absolute growth rate is proportional to plant size, relative growth rates allow a comparison of the physiological efficiency of different genotypes and can help to explain how a given genotype behaves in distinct environments.

Relative growth rate. This measures the average efficiency of each unit of dry matter in the rate of production of new dry matter (Causton & Venus 1981) and is given by the following expression:

$$RGR = (1/W) \times (dW/dt).$$

The mean RGR during the period $t_1 - t_2$ is calculated by the equation:

$$RGR = (\log_e W_2 - \log_e W_1) / (t_2 - t_1).$$

Again the only necessary assumption is that W varies without discontinuity throughout the period $t_1 - t_2$ (Radford 1967).

Unit leaf rate, also called the Net Assimilation Rate. This measures the rate of dry matter production per unit of leaf area and is given by the following equation:

$$E_A = (1/L_A) \times (dW/dt),$$

where L_A is total leaf area at time t (Causton & Venus 1981). This is an approximate measure of net photosynthetic rate if mineral ion uptake is ignored (ibid.). In effect, E_A estimates the rate of carbohydrate output from the photosynthetic system minus the loss due to respiration.

The mean E_A during the period $t_1 - t_2$ is calculated by the equation:

$$E_A = [(W_2 - W_1)/(A_2 - A_1)]*[(\log_e A_2 - \log_e A_1)/(t_2 - t_1)].$$

There are two assumptions involved in the use of this mean E_A: 1) A and W are linearly related over the period $t_1 - t_2$; and 2) A and W are not discontinuous functions of time (Radford 1967). If A and W are not linearly related, E_A is not estimated correctly; the larger the deviation from linearity, the larger the error of estimation (ibid.). Thus, it is important to explore the relationship of A with W before embarking on the use of

formulas to estimate mean E_A. Radford (1967) presents several alternatives for different relationships between A and W.

Leaf area ratio. This measures the amount of dry matter invested in the production of more dry matter, i.e. the partitioning of dry matter to the photosynthetic system, and is given by the following equation:

$$LAR = L_A/W$$

Colloquially, the leaf area ratio is an index of the plant's 'leafiness' (Causton & Venus 1981). The formula normally used to estimate mean LAR during the period t_1 - t_2 is:

$$LAR = [(L_{A1} / W_1) + (L_{A2} / W_2)] / 2.$$

The assumption involved in its use is that LAR is linearly related with time (Radford 1967).

Unit leaf rate and leaf area ratio are intimately related as components of the relative growth rate, the equation of which can be rewritten as:

$$RGR = E_A \times LAR.$$

This is an extremely important relationship, because it states that a plant may have a high RGR either because of a high E_A or a high LAR, though not usually both (Causton & Venus 1981). Thus, a high leaf area in relation to overall plant weight (LAR), combined with a moderate net photosynthetic rate ($\approx E_A$), can make for a highly efficient plant, as can the reverse properties. Both of these quantities present heritable variation in plants, although Simmonds (1979) reports that little genetic gain had been achieved by the mid-1970s and Gupta (1992) essentially confirms this in the late 1980s. This is probably because these traits are extremely complex polygenic systems, which may depend upon

finely balanced linkage equilibria among blocks of genes (Gupta 1992), and also are strongly influenced by environmental variation.

2.6.1. Growth Analysis in African Oil Palm

Economic yield of a crop is equal to its biological yield, measured as the sum of vegetative and reproductive dry biomass, multiplied by the Harvest Index (HI) (Donald 1962). A crop with a high HI partitions a greater fraction of photoassimilates to economic yield (Rees & Tinker 1963, Hartley 1977, Corley 1983) and has a smaller growth habit (Gifford et al. 1984). This demonstrates the potential importance of growth and physiological analysis in plant improvement. The growth and physiological parameters of interest in palms are:

Yield (kg/plant/yr). In oil palm, yield is the total dry weight of the fruit bunches (Corley & Gray 1976b). In the case of pejibaye for heart-of-palm, yield will be total weight of the export quality heart, plus edible stem and leaves that have potential market value (Clement et al. 1993).

Vegetative Dry Matter (kg/plant/yr). In oil palm, this is the total dry weight of the leaves produced during the year plus the annual trunk increment (Corley & Gray 1976b). In contrast to oil palm, yield of pejibaye for heart-of-palm is a sub-component of the vegetative growth, rather than a separate component.

Crop Growth Rate (t/ha/yr). In oil palm, CGR is the sum of fruit yield and vegetative dry matter production, multiplied by the number of plants/hectare (Corley & Gray 1976b). In the case of pejibaye for palm heart, CGR is vegetative dry matter production.

Harvest Index (HI). The proportion of economic product (as dry biomass) to vegetative dry matter produced. In oil palm, this is the oil extracted from the fruit bunches. In pejibaye for heart-of-palm, this will be the dry weight of the heart as a fraction of the vegetative dry matter produced.

Leaf Area Index (LAI). The Leaf Area Index is the leaf area per unit of land in the plantation. It is calculated by: LAI = whole plant leaf area ÷ area occupied by the plant. In oil palm, plant leaf area and LAI are highly correlated with yield (Hardon et al. 1969).

Unit Leaf Rate (E_A). This is used precisely as defined by Radford (1967)

Leaf Area Ratio (LAR). LAR is the new leaf area produced during a period, generally the year. This is slightly different from Radford's (1967) definition, in that only new leaf area is included in the calculation, because oil palm leaves remain photosynthetically active for more than a year.

2.6.2. Growth Analysis in Pejibaye

Gutierrez et al. (1986) estimated LAI and E_A in the Panamá germplasm collection at CATIE (Occidental complex). They found as much variation in that one population as has been reported from African oil palm.

Moreira Gomes & Arkcoll (1988) found that, in Pará landrace germplasm, heart-of-palm weight increases with height up to approximately four m, after which it plateaus. Unfortunately, they did not specify how they measured height, so no regressions between height and weight can be made *post facto*. They also showed that heart-of-palm yields increased with increasing CGR, while individual heart weight decreased (Table 2.2).

Although their data is from a small number of plants, it is clear that pejibaye responds to high densities in much the same way as African oil palm.

Table 2.2. Effect of density on Pará landrace pejibaye growth and heart-of-palm yield on a nutrient poor Oxisol in Central Amazonia (Moreira Gomes & Arkcoll 1988). All plants received 100 g NPK (equal amounts) in the first year and 200 g in each subsequent year.

Density (plants/ha)	Heart Yield (kg/ha)	Heart Weight (g/plant)	Crop Growth Rate (t/ha/yr)
1600	401	251	4.8
2500	605	242	8.1
4444	880	200	13.2

Moreira Gomes et al. (1988) established a density x fertility trial on poor Oxisols in Ouro Preto d'Oeste, Rondônia, Brasil, using Putumayo landrace germplasm. Yield increased with density, while heart weight generally decreased (Figure 2.4). There was also a pronounced effect of fertilizer on both yield and heart-of-palm weight.

Zamora (1985) reported on an interesting density trial in Costa Rica, with local germplasm. The results, however, are difficult to interpret because he reported yields in terms of field ready heart-of-palm, rather than export quality heart-of-palm. Somewhere between 20 and 30% of the field ready stem is export quality and the percentage may vary with density. The yield curve (Figure 2.5) is more peaked than expected, with the 5000 plants/ha density yielding 500 kg more than the next higher or lower density. The decrease in the number of palms harvested as density increases reflects competition. The Occidental pejibaye germplasm has heavier leaves and somewhat larger leaf area than the Amazonia germplasm, which may explain the rapidity with which the % cut decreased in Zamora's experiment. The % cut is one measure of precocity, since plants must attain

harvest size to be cut. The lower % cut reflects slower growth of plants in the plot, probably because of competition. This well designed experiment would have benefited enormously from the use of growth analysis.

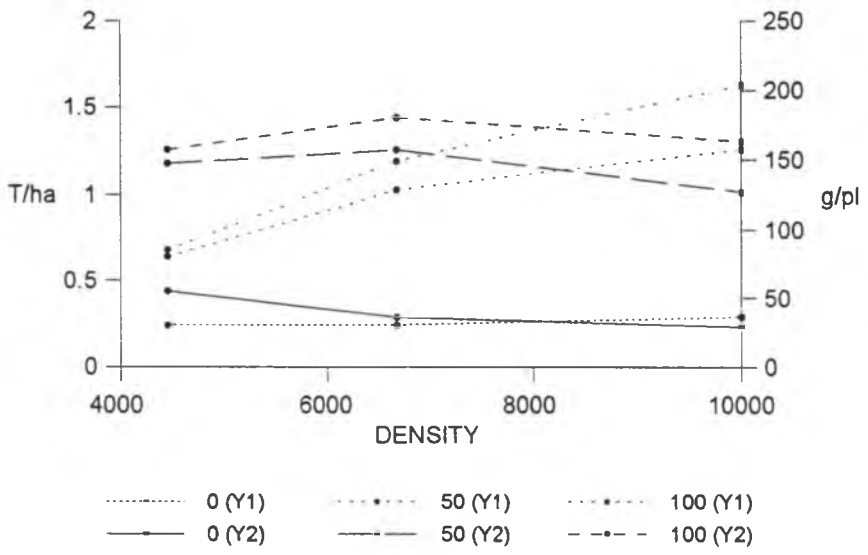

Figure 2.4. The effect of density and fertilization on Putumayo landrace pejibaye heart-of-palm yield (axis Y1) and individual heart-of-palm weight (axis Y2) on a nutrient poor Oxisol in SW Amazonia (Moreira Gomes et al. 1988). Fertilization: 0 g/plant of N, P, and K; 50 g of each; 100 g of each.

2.7. Genetic Analysis

> "The genetics of a metric character centres round the study of its variation, for it is in terms of variation that the primary genetic questions are formulated. The basic idea in the study of variation is its partitioning into components attributable to different causes. The relative magnitude of these components determines the genetic properties of the population, in particular the degree of resemblance between relatives." Falconer (1981: 112).

In plant breeding, quantitative genetics offers a guide to the plant breeder, although this guide is frequently only a confirmation of the breeder's intuitive sense of which traits can be easily modified in a breeding program and which will be more difficult

(Simmonds 1979). As suggested by Falconer (1981) in the above quotation, the study of quantitative genetics is the study of variation, specifically the statistical variation observed in natural or, principally, experimental populations.

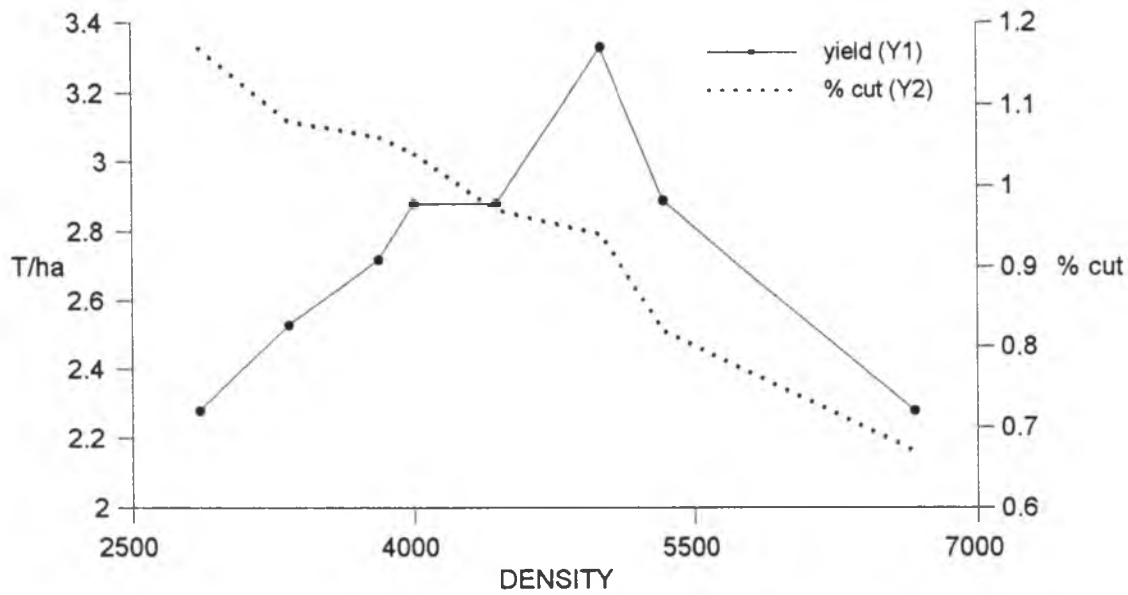

Figure 2.5. The effect of density on Costa Rican pejibaye field-ready heart-of-palm yield (axis Y1) and the percentage of plants in each plot that are harvested each year (% cut: 1 = 100%; axis Y2) on a nutrient rich Alfisol in Costa Rica.

The partitioning of this experimental variance into components attributable to different causes is the basis of quantitative genetics and allows the plant breeder to confirm his intuitive sense of how much heritable variation is present and how it can be manipulated. The genius of the early biometrical geneticists, principally R.A. Fisher, J.B.S. Haldane and S. Wright, was in identifying which components of variance could be attributed to a given genetic parameter of the experimental population (Simmonds 1979, Falconer 1981). Their assumptions have been widely tested and generally confirmed by

several generations of quantitative geneticists, plant breeders and applied evolutionists since their formulation in the 1920-30s.

It must always be emphasized that the phenotypic variation in a given experimental population is a function of the underlying gene and allele frequencies of the population sample and the environment in which the population develops (Falconer 1981, Nyquist 1991). Thus, any estimates of genetic variances and parameters are specific to the population sample in the environment in which it is studied (Falconer 1981). Nyquist (1991) emphasizes the environmental component of these estimates, sub-dividing it into the micro-environments in which each individual plant develops and which is not under the control of the researcher, and the macro-environment in which the experiment is carried out and which is under the control of the researcher. This emphasis highlights the existence and importance of genotype by environment interactions, which are exceptionally important in plant breeding.

Additionally it must be emphasized that the analysis of experimental variation is a statistical process, utilizing specifically the variances of the traits being studied (Simmonds 1979). In general, variances are much less statistically robust than are means and standard deviations, so that the estimation of these variances is inevitably less precise and contains greater possibility of error than the estimation of means (Simmonds 1979, Nyquist 1991). Consequently it is not uncommon to encounter negative variances and the standard errors of the heritabilities are often as large or larger than the heritabilities themselves.

2.7.1. Genetic Analysis in Economically Important Palms

All improvement plans are designed to maximally exploit the available genetic diversity and the heritability of those characters of economic importance (Simmonds 1979). In perennials like oil palm, coconut and pejibaye, these plans must be as efficient as possible because generations are long, it is very expensive to plant and maintain the experiments, and it is very rare that a trial can be planted only to estimate the heritabilities that will help define the most adequate methodology (Hardon 1976).

All the improvement programs in African oil palm and coconut started with mass selection of open-pollinated populations (Liyanage 1972, Child 1974, Hardon 1976, Hartley 1977). With open pollination, however, it is only possible to select one parent, so that genetic gain is relatively slow (Liyanage 1972), unless the heritability is very high. Nonetheless, all new germplasm added to improvement programs from the germplasm banks is initially selected from open-pollinated progenies.

Thereafter, both oil palm and coconut breeders started making controlled crosses between only a very few selected parents, followed by progeny tests (Liyanage 1972, Child 1974, Hardon 1976, Hartley 1977). This methodology is much more labor-intensive than pure mass selection, but permits more rapid advance (Simmonds 1979). According to Hardon (1970) this is the most efficient way to exploit the additive genetic variance present in the base populations that originated the oil palm improvement programs in Malaysia and Asia in general. Due to the small number of parents that can be used in each cycle, however, a rapid narrowing of the genetic base of the improved

populations is inevitable. Therefore it is extremely important to add new germplasm at each cycle, which also increases the additive genetic variance available.

In Africa, Sparnaaij (1969) and Sparnaaij et al. (1963) found larger gains in between- than in within-population hybrids, so the French oil palm breeders started using inter-population crosses with the expectation of obtaining more rapid advances through heterosis. Given the biological characteristics of the oil palm, the reductions in yield observed from inbreeding, and the low heritabilities for yield and variable heritabilities for other fruit and bunch characteristics, it is clear that crosses between unrelated populations with complementary characteristics should give better yields through heterosis (Meunier & Gascon 1972). The French workers systematized a methodology based on reciprocal recombinant selection, combined with progeny tests (Meunier & Gascon 1972). These workers claim that this methodology is more efficient because it exploits both additive and non-additive genetic variances and it allows a clear evaluation of both general and specific combining abilities.

In the French plan, base populations are formed that concentrate important economic and physiological characteristics. For example, one population will concentrate genes for large bunches, another genes for large bunch number (these two characteristics are highly and negatively correlated, so they must be concentrated in different base populations (Hartley 1977)). With these initial populations two types of crosses are made in a two step sequence: 1. the best plants from population 1 are crossed with the best from population 2, followed by progeny tests to classify the parents with respect to combining ability; 2. the plants that give the best results are self-pollinated and crossed within their

respective base populations, thus forming inbred populations for seed production and an improved generation of the base population, respectively. The hybrids produced from the inbred populations regain vigor and frequently exhibit heterosis (Jacquemard et al. 1981).

In both improvement plans, the progeny tests allow the selection of outstanding individuals, outstanding parents and outstanding families, so that there is efficient selection at three levels within the program (Ooi 1978). Logically, the response to selection, or genetic gain, is primarily a function of the heritabilities of the traits selected.

Yield generally has low heritabilities (Allard 1960, Simmonds 1979) in both the broad- and the narrow-sense, and this is certainly true in oil palm, where estimates vary from extremely low (0.04) to medium (0.53) at best (Hardon et al. 1972, Ooi 1978, Breure & Corley 1983).

Work by Hardon et al. (1972), Tan (1978) and Breure & Corley (1983) showed that many of the growth and physiological parameters have medium to high heritabilities and are therefore more easily modified by selection. These three studies were carried out in environments similar to those found on the Hamakua coast of the Big Island and some areas in tropical Latin America where pejibaye is being planted commercially. Since pejibaye has been shown to be quite similar to oil palm in several aspects of growth and morphology, as well as presenting as much or more phenotypic variation, it is expected that pejibaye will present narrow-sense heritabilities within the range of magnitudes shown in Table 2.3 for oil palm.

Table 2.3. Narrow-sense heritabilities of some growth and physiological parameters studied in *Elaeis guineensis*. Each row presents results of a different trial.

Reference	VDM	CGR	LAR	LAI	E_A	L_A
Hardon,				0.29	0.34	
Corley & Ooi	0.65	0.56		1.15	0.27	1.07
(1972)	0.41		0.32		0.17	
Tan (1978)	0.24	0.06	0.83	0.12	0.21	0.13
Breure &	0.63	0.65	0.68		0.55	0.85
Corley (1983)	0.21	0.53	0.66		0.32	0.69

Breure & Corley (1983) suggested that a combination of high LAR and BI (Bunch Index = Bunch weight/(yield + vegetative dry matter), a purely reproductive measure of HI) would be useful in identifying plants and progenies that demonstrate tolerance to competition. Hartley (1977), Hardon (1976) and Ooi (Ooi 1978) affirm that most selections have been made (unintentionally) for competitive plants, because high yield was sought without considering LAI, LAR or BI. These plants are good individual yielders (and competitors) but, when planted in uniform populations, often yield less than expected. This explains why many modern plantations show a reduction in yield after the canopy closes. The combination of LAI, LAR and BI allows the selection of plants that are good yielders, tolerant of competition, and generally smaller. This allows higher density and thus greater yield on a per hectare basis.

Clement & Mora Urpí (1988) demonstrated that there is considerable variation within and among pejibaye populations for leaf area, and, consequently, for LAI. Similar variation should exist among progenies within populations as well. Given the similarities

observed in growth of pejibaye and oil palm, the genetics of growth in oil palm should be comparable to those in pejibaye also.

2.8. Plant Isoenzymes

During the last several decades, the use of isoenzymes in plant improvement has expanded rapidly, as has its use in numerous other areas of the biological sciences (e.g., Tanksley & Orton 1983a, b, Soltis & Soltis 1989, Khanna 1991). Isoenzymes have become such popular tools because they generally exhibit Mendelian inheritance, co-dominant expression, complete penetrance and an absence of pleiotropic and epistatic interactions (Weeden & Wendel 1989), all of which facilitates their genetic analysis. Because many of them are components of physiological processes, they may also serve as markers for those quantitative traits related to growth and yield (Stuber 1991, Koutou et al. 1992). In fruit tree improvement isoenzyme analysis has served several useful functions, including characterization and identification of cultivars, discrimination of selfed versus crossed progeny, determination of the genetic origin of seedlings, documentation of the parentage of cultivars, and examination of the similarities among cultivars (Torres 1989).

2.8.1. Isoenzymes in Palms

The first isoenzyme study carried out in the Palmae was done on date palm (*Phoenix dactylifera*) by Torres & Tisserat (1980). Five enzyme systems were used to discriminate among cultivars. None of these five systems correlated with sex, however, which would

have been the most useful marker in that species. French and Arab researchers have used isoenzyme analysis to evaluate genetic diversity of date palm in Algeria (Table 2.4) (Brac de la Perrière 1988, Brac de la Perrière & Ben Khalifa 1989, Bennaceur et al. 1991).

Table 2.4. Some examples of allozyme variability in economic palms.

	Enzyme	Polymorphic	Alleles/	Observed Heterozygosity	
Species	Systems	Loci	Locus	minimum	maximum
P. dactylifera[a]	7	7	2.3	0.40	0.50
E. guineensis[b]	9	13	2.3	0.18	0.37
E. oleifera[c]	13	11	2.1	0.12	0.29

[a] (Bennaceur et al. 1991); [b] (Ghesquière 1985); [c] (Ghesquière et al. 1987)

In African oil palm, Ghesquière (1984, 1985) determined the genetics of 9 pollen enzyme systems and used this to characterize the variability and genetic structure of 7 oil palm provenances used in the French improvement program in the Ivory Coast (Table 2.4). Hutomo & Subronto (1991) examined 21 enzymes, finding 23 loci and 56 alleles, to study genetic variability in oil palm germplasm in Indonesia. Ghesquière et al. (1987) used seedling leaf enzymes to evaluate the possible relationships among populations of the American oil palm (*Elaeis oleifera*) collected in Brazilian Amazonia (Table 2.4). Santos & Mestriner (1989) refined the extraction process from mature oil palm leaves. Santos (1991) determined the probable relationship among the Bahia state (Brazil) subspontaneous populations (once thought to be indigenous to South America) and suspected parental stocks in Angola and Zaire.

Rojas Vargas (1993) succeeded in resolving 10 enzyme systems (ADH, ACP, DIA, EST, MDH, ME, PGI, PGM, PRX, SOD) from small (n = 5) samples of five pejibaye

populations (Darien, Guapiles, Chaparé, Belém, Yurimaguas). Not surprisingly, leaf tissue proved to be the most satisfactory tissue for extracting enzymes. Miranda (1993) extracted enzymes from pollen of the Yurimaguas population and used it to determine the relationships among five plants.

Table 2.5. Enzymes extracted from pollen or leaf tissue of date palm (*Phoenix dactylifera*), African oil palm (*Elaeis guineensis*), macaúba (*Acrocomia aculeata*), and pejibaye (*Bactris gasipaes*) for electrophoretic analysis in previous studies.

Enzyme	Abrev.	*Phoenix*	*Elaeis*	*Acrocomia*	*Bactris*
Phosphoglucoisomerase	PGI	+	+	+	+
Phosphoglucomutase	PGM	+	+		+
Malate dehydrogenase	MDH	+	+	+	+
Malic enzyme	ME				+
Shikimate dehydrogenase	SKDH	+	+		
Alcohol dehydrogenase	ADH	+			
Leucine aminopeptidase	LAP	+			
Glucose-6-phosphate	G6PDH				+
Phosphogluconate dehydrogenase	PGD		+		
Amylase	AMY		+		
Aspartate aminotransferase	AAT	+			
Diaphorase	DIA	+			+
Acid phosphatase	ACP	+	+		+
Isocitrate dehydrogenase	IDH	+	+		
Peroxidase	PER	+		+	+
Esterase	EST	+		+	+
Superoxide dismutase	SOD				+
Hexokinase	HK	+			
Aconitase	ACO				+
Endopeptidase	ENDO	+	+		

2.8.2. Correlations of Isoenzymes with Other Traits

Especially interesting in the context of this project is the correlation of allozyme heterozygosity with growth in trees. Reviews by Mitton (1989) and Bush & Smouse (1992) strongly suggest that heterogeneity at several common enzyme loci is correlated with mean growth rate in pitch pine (*Pinus rigida*) (citing Ledig et al. (1983)), and knobcone pine (*Pinus attenuata*) (citing Strauss (1986)), especially at higher densities where competition is more intense. Aradhya & Phillips (1995) did not find a correlation between heterozygosity at eight polymorphic loci and seedling growth in four *Eucalyptus* species. Stuber (1991) emphasizes that the positive correlations are more pronounced at high levels of competition, which suggests that good competitors have high levels of heterozygosity and that this may be equated with heterosis (Mitton 1989, Stuber 1991). Bush & Smouse (1992) point out that increases in population heterozygosity over time imply superior survival of heterozygotes and for forest trees, a large component of survival is *the capacity for early growth* (citing Spurr & Barnes (1980)) (emphasis added). The available data indicate that there is a relationship between allozyme heterozygosity and early growth that would be worth examining in pejibaye.

Chapter 3. Material and Methods

3.1. Germplasm

Ten open-pollinated progenies of the Benjamin Constant population of pejibaye were obtained from the National Research Institute for Amazonia (INPA), Manaus, Amazonas, Brazil, in April of 1991 (Table 3.1). The parental plants are maintained in a 6-species agroforestry trial at the Tropical Fruit Experiment Station, 60 km north of Manaus. The germplasm used in this agroforestry trial came from the Benjamin Constant (Amazonas, Brazil) population of the Putumayo "macrocarpa" landrace from northwestern Amazonia (Mora Urpí & Clement 1988). Seed was selected from parents with spineless stems and leaf petioles and free of phytosanitary problems. No other selection was practiced. In the agroforestry experiment about 80% of the plants are spineless (W.B. Chávez F., 1990, personal communication).

The history of this germplasm is worth examining in detail, because this project found strong evidence of significant inbreeding (Chapters 6 & 7). The majority of the progenies (the exception being B-9) came from a seed orchard that had been formed as the result an expedition to Benjamin Constant, Amazonas, Brazil, by Dr. Platón, of Belém, Pará, Brazil, in the late 1950's. The objective of the expedition was to find spineless-stemmed pejibaye. Since the Benjamin Constant population contains 10-15% spineless plants, only this sub-population was sampled at that time, significantly reducing the genetic variability taken to Dr. Platón's seed orchard, in Santa Isabel de Pará. The seed orchard contained about 200 plants, all the result of that single expedition. Unfortunately, Dr. Platón died in 1974, without leaving documentation for the seed

orchard. Consequently, the number of plants sampled in Benjamin Constant remains unknown, but is not likely to have been very large.

Table 3.1. Identification information on seed of the Benjamin Constant pejibaye population received in April 1991.

U.H. Identification	INPA Identification	Date Collected	Seeds Received	Phyto Status*
B-0	INPA-1.8.2	05 Mar 91	400	5
B-1	INPA-3.1.9	05 Mar 91	610	1
B-2	INPA-3.1.10	05 Mar 91	639	5
B-3	INPA-3.1.11	22 Mar 91	397	2
B-4	INPA-3.4.1	08 Apr 91	473	1
B-5	INPA-3.4.2	21 Mar 91	400	3
B-6	INPA-3.4.4	22 Mar 91	399	1
B-7	INPA-3.4.6	08 Apr 91	407	1
B-8	INPA-3.8.16	22 Mar 91	391	1
B-9	INPA-5.5.3	22 Mar 91	326	5

* Phytosanitary Status on arrival in Hawai'i: 1 - excellent, only a few seeds with visible fungus; 5 - poor, more than 10% of the seeds with visible fungus.

When Dr. Platón's seed orchard was sampled in 1976 by INPA, it contained only spineless plants, suggesting that nursery selection against spines had been practiced, resulting in further reductions in the genetic variability of the orchard. The 1976 sample selected only 10 spineless-stemmed plants, although with variable fruit characteristics, resulting in a further reduction in the genetic variability in the agroforestry trial planted in Manaus in 1977. Approximately 400 seedlings from this sample were planted, without maintaining records of the maternal plants.

Progeny B-9 was obtained directly from Benjamin Constant by Dona Eva van der Pahlen, INPA, in 1976. The parent tree was also selected for spinelessness and only spineless seedlings were planted in the agroforestry trial. Again, no record of the maternal germplasm was maintained.

3.2. Germination

All seed were soaked in fresh water for one hour after arrival and the number that floated was counted. Seed that sink were considered potentially viable, while those that floated were not (Copeland & McDonald 1985). All seed were cleaned, treated with Captan, and re-bagged.

The germination substrate was a mixture (v:v) of three parts redwood bark chip (Big R) and two parts horticultural perlite (# 2). Ten cm of this substrate was placed in galvanized nursery flats (60 L x 40 W x 10 D cm), with two 5-minute sprinkler irrigations per day, at the Magoon Nursery, Honolulu. The seeds were sown in lines, with 2 cm between seeds and 5 cm between lines, at a depth of 1 cm, on 25 April 1991.

The germination experiment was a split-plot, with greenhouse environments (20% shade cloth vs. glasshouse) as the main plots, and progenies (10) as the sub-plots, with four replicates in each environment and 25 seed per plot. The flats were observed weekly and the number of plants germinated in each plot was counted. A month after no more seed germinated (21 August 1991), the seedlings were transplanted to pots in the nursery.

3.3. Nursery Conditions

The potting substrate was a mixture of three parts redwood bark chip (Big R) and two parts horticultural perlite (#2), with 1.4 kg of Nutrite (14N-6P-11.6K, elemental concentrations) 3-6 month slow-release fertilizer and 1 kg Ag 10 coarse-ground dolomite per m^3 of substrate. Nutrite proved to be an inadequate fertilizer, so Osmocote (17N-2.6P-10K, with minors) 3-6 month slow-release fertilizer was used as a top dressing after three months with good results.

The potting substrate was put into 4- x 4- x 10-inch bottomless plastisized-paper planting-sleeves (Monarch) placed in galvanized wire racks (12 sleeves per rack). The paper sleeves are essentially discardable, inexpensive dibble tubes and shape the root system similarly. Because they are square-cornered, plant roots growing laterally will contact a side, turn laterally to a corner, then turn down to the bottom, where they will be air-pruned.

At transplanting, the seedlings were held in 50% shade for two weeks, then maintained in 20% shade for two more weeks in the screenhouse, all the time receiving two 5-minute sprinkler irrigations per day. They were then transfered to full sun, where they received two 10-minute sprinkler irrigations per day.

The plants were field ready by February 1992. Since an El Niño drought was occurring at this time, they were held at Magoon until June 1992, when they were shipped to their experimental sites for planting.

3.4. Localities

The localities chosen represent three of Hawai'i's important agricultural areas: the Hamakua coast, Island of Hawai'i, historically important for sugarcane; the Ola'a lava flows south-east of Hilo, Island of Hawai'i, the major papaya district; and north-central Oahu, historically important for sugarcane and pineapple. All three areas are currently changing rapidly because of the demise of the sugarcane plantations and the recent introduction of papaya ringspot virus, and will require new crops to remain agriculturally important areas.

3.4.1. Ninole

The farm of Mr. John Mood, Ninole, Island of Hawai'i, is on the lower slopes of Mauna Kea. Ninole is 20 miles north of Hilo on route 19. Latitude is 19°56'8" N and longitude is 155°11'34" W. Altitude is approximately 160 m above sea level (a.s.l.). A density x progeny trial for each population was planted at this site.

The soil is classified as an Andisol, Hilo Series, silty clay loam. The Hilo Series is a Typic Hydrudand, hydrous, isohypothermic soil, with pH ranging from 4.5 - 6, and slopes from 0 - 10%. The pH at the site was 5.4, with 28 kg/ha of phosphorous (14 ppm), 45 kg/ha of potassium (22 ppm), 555 kg/ha of calcium (277 ppm) and 275 kg/ha of magnesium (137 ppm) in the plow horizon soil at the beginning of the experiment. Appendix 1 contains a full description of a representative profile of the series.

The climate at Ninole is classified as 'Af' in the Köppen system (Juvik et al. 1978, Sanderson 1993), with an average of 3,479 mm of rain and a mean annual temperature of

25°C. Figure 3.1 contrasts the 20-year monthly rainfall mean at Honomu (5 miles southeast of Ninole) with the recorded rainfall during the experimental period at Ninole.

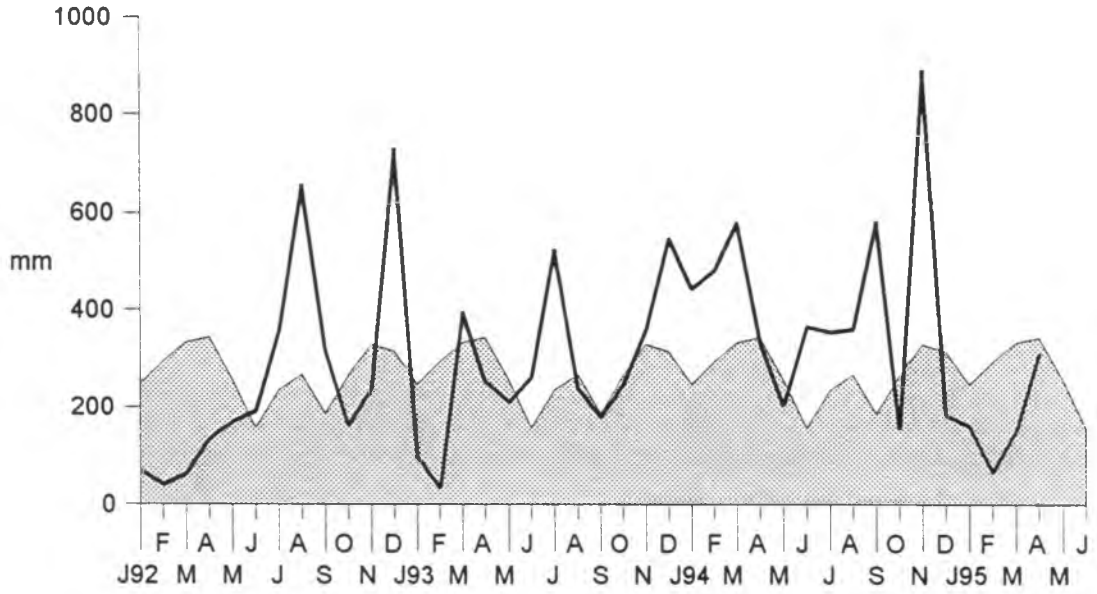

Figure 3.1. The 20-year mean rainfall at Honomu, HI, Island of Hawai'i, 8 km SE of Ninole at the same elevation (shaded area), and the recorded rainfall during the experimental period at Ninole.

3.4.2. Waiakea

Waiakea Experiment Station, Hilo, Island of Hawai'i, is on the lower slopes of Mauna Loa. Latitude is 19°38'25" N and longitude is 155°6'0" W. Altitude is approximately 175 m a.s.l. This site received a progeny trial at 5000 plants/ha density.

The soil of the experimental field is classified as an Histosol, Keaukaha Series, very stony muck. The Keaukaha series is a Lithic Tropofolist, dysic, isohypothermic soil, with pH ranging from 5-6, and slopes of 0 - 5%. The pH at the site is 5.6, with 35 ppm of phosphorous, 40 ppm of potassium, 500 ppm of calcium and 100 ppm of magnesium in the soil at the beginning of the experiment. (The nutrient elemental status of the

Keaukaha soil is not given in kg/ha because there is no plow horizon.) Appendix 1 contains a full description of a representative profile of the series.

The climate at Waiakea is classified as 'Af' in the Köppen system (Juvik et al. 1978, Sanderson 1993), with an average of 4802 mm of rain and a mean annual temperature of 25°C. Figure 3.2 contrasts the 20-year monthly rainfall mean at Waiakea with the recorded rainfall during the experimental period.

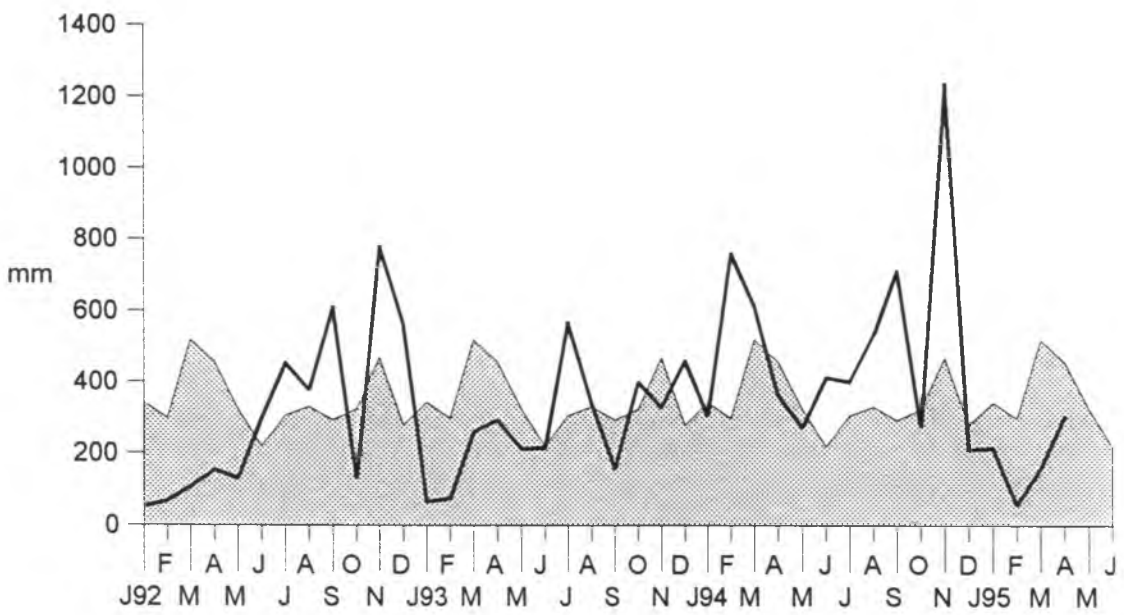

Figure 3.2. The 20-year mean rainfall at Waiakea Experiment Station, Hilo, HI, Island of Hawai'i (shaded area) and the recorded rainfall during the experimental period.

3.4.3. Poamoho

Poamoho Experiment Station is up-hill from Waialua, Island of Oahu. Latitude is 21°32'30" N and longitude is 158°05'15" W. Altitude is approximately 175 m a.s.l. The experimental plot had previously been used to study several seed legumes. Pejibaye was

planted in the late 1970s at Poamoho and did well where adequately tended. This site received a progeny trial at 5000 plants/ha density.

The soil of field L is classified as an Oxisol, Wahiawa Series, silty clay. The Wahiawa series is a Rhodic Eutrustox, clayey, kaolinitic, isohyperthermic soil, with pH ranging from 4.5 - 6, and slopes from 0 - 5%. The pH at the site is 5.8, with 25 kg/ha of phosphorous (12 ppm), 40 kg/ha of potassium (20 ppm), 2000 kg/ha of calcium (1000 ppm) and 500 kg/ha of magnesium (250 ppm) in the soil at the beginning of the experiment. Appendix 1 contains a full description of a representative profile of the series.

The climate at Poamoho is classified as 'Aw' in the Köppen system, with an average of 1078 mm of rain and a mean annual temperature of 26.5°C. Figure 3.3 contrasts the 20-year monthly rainfall mean at Poamoho, with the recorded rainfall during the experimental period.

3.5. Plantation Densities

a. Commercial planting density in Costa Rica is 5,000 plants/ha, using a planting geometry of 1 x 2 m (Mora Urpí et al. 1984). This density and geometry was used at all sites in the current study and was the density of reference.

b. 6,666 plants/ha; geometry of 1 x 1.5 m.

c. 3,333 plants/ha; geometry of 1.5 x 2 m.

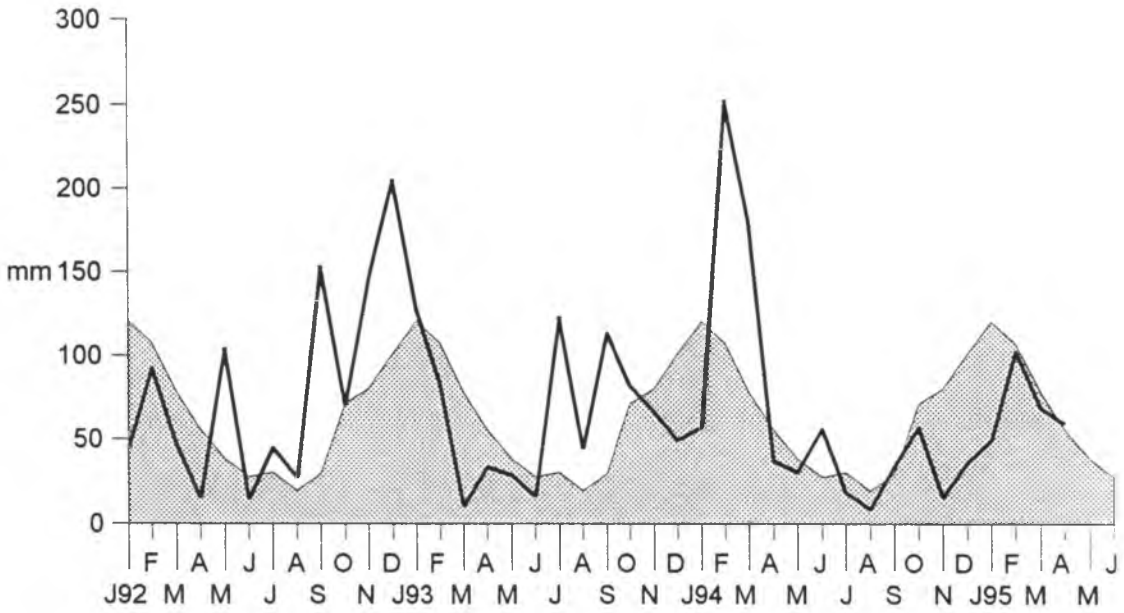

Figure 3.3. The 20-year mean rainfall at Poamoho Experiment Station, Waialua, HI, Island of Oahu (shaded area) and the recorded rainfall during the experimental period.

All three densities, with their respective geometries, have been used in experiments in Latin America. Additionally, the higher and lower densities bracket the reference density by an equal number of plants (1,666). Because of limited planting materials, however, each plot is only three plants wide, which means that the effect of density may not be very pronounced. The lower and higher densities were used only at Ninole.

3.6. Experimental Designs

a. Ninole

The experimental design at Ninole was a split-plot, with densities (3) as the main plots and progenies (7) as the sub-plots, with 3 replications and 9 plants/plot. The lower density (3333 plants/ha) had 7 progenies (0, 1, 2, 3, 5, 8, 9), the higher density (6666

plants/ha) had 8 progenies (as before, plus 6), the reference density (5000 plants/ha) had 9 progenies (as before, plus 6 + 7). Consequently, the basic design was based on 7 progenies. Total number of plants in the field was 648. The following ANOVA outline shows the basic design (Little & Hills 1978). The augmented progenies were treated according to Brewbaker (1995). The components of variance for genetic analysis are presented following Brewbaker (1995). ANOVAs were done with the GLM routine of Minitab 10.2. Least significant differences were calculated as $t_{(0.05)} \cdot [(MS_{error\ b})/(rep*main\ plots)]^{½}$ (Little & Hills 1978).

Source of Variation	df	components of variance
Replications	2	
Main plots		
Densities	2	
Main plot error (a)	4	
Sub-plots		
Progenies	6	Vc + sVrdp + srVdp + srdVp
Progenies x Densities	12	Vc + sVrdp + srVdp
Sub-plot error (b)	36	Vc + sVrdp
Sampling error (c)	504	Vc
Total	566	

b. Waiakea & Poamoho

The experimental design used at Waiakea and Poamoho was a randomized complete block, with 3 replications and 9 plants/plot. At Waiakea, progenies 1, 2, 3, 6, 8, 0 occured in all three blocks, progenies 5 and 9 occured in blocks 1 and 2, and progeny 4 in block 3. Total number of plants in the field was 207. At Poamoho, progenies 1, 2, 3, 5, 6, 8 occured in all three blocks, and progeny 9 had only six plants in blocks 1 and 3, and 9 in block 2. Total number of plants in the field was 184. All plants were at 5,000 plants/ha

density. Consequently, the basic design was based on 6 progenies. The following ANOVA outline shows the basic design (Little & Hills 1978); the components of variance for genetic analysis are presented following Brewbaker (1995).

Source of variation	df	components of variance
Replications	2	
Progenies	5	V + sVrp + srVp
Exp. error	10	V + sVrp
Sampling error	144	V
Total	161	

c. Combination of locations

The 5,000 plants/ha density appeared at all locations and were combined into one analysis. The design was a multi-location randomized complete block, with three locations, three replications per location, and five progenies (1, 2, 3, 6, 8). The following ANOVA outline shows the basic design (Cochran & Cox 1957, McIntosh 1983); the components of variance for genetic analysis are presented following McIntosh (1983), adjusted for sampling error.

Source of variation	df	components of variance
Locations	2	
Replications in Locations	6	
Progenies	4	Vc + sVb + srVlp + srlVp
Progenies x Locations	8	Vc + sVb + srVlp
Error (b)	24	Vc + sVb
Sampling error (c)	360	Vc
Total	404	

3.7. Field Preparation and Planting

a. Ninole.

Site preparation involved deep plowing, disking, liming (1 Mt/ha of coarse ground dolomite), and roto-tilling. The Soil Conservation Service sited water management contours immediately above and below the experimental field. Field planting was originally scheduled for February 1992, but was postponed because of the El Niño drought event of that period (Figure 3.1). By May, reasonable rainfall had returned to the Hamakua coast, and the Ninole site was planted on 30-31 May 1992. The next two months had irregular rainfall, however, so plants were watered occasionally.

b. Waiakea.

Site preparation involved breaking up the lava and grading to cut hills and fill holes, leaving a very flat site, but with irregularly distributed soil. Planting at Waiakea was done on 7 August 1992. Plants were watered in, but rainfall was reasonable during the planting period so they only received one watering.

c. Poamoho.

Site preparation involved plowing and disking. The planting holes were augered with a tractor-mounted 25-cm-diameter auger to a depth of 50 cm. Irrigation furrows were prepared before planting, with one furrow immediately adjacent to each row of plants. Planting at Poamoho was done on 30 July 1992.

All plants received 200 g Osmocote (17N-2.6P-10K, with minors) 3-6 month slow-release fertilizer and 100 g Ag-10 coarse-ground dolomite in the planting pit.

3.8. Field Management

Replanting was done as necessary during the months after planting. Fifteen plants were substituted at Ninole (2.3% replacement), 22 at Waiakea (10.2%), and 15 at Poamoho (5.7%). The high replacement number at Waiakea was due to several short droughts in late 1992, which killed plants that had been considered as established. The moderate replacement number at Poamoho was due to temporary manpower difficulties at the station that reduced irrigation frequency from once a week to once in two weeks; irrigation frequency returned to once a week from 6 months after planting to present.

At Ninole and Waiakea, three-foot-wide, black, woven polypropylene weed mat was installed around the plants after establishment. This was not used at Poamoho because weed populations were smaller and the irrigation furrows were very close to the plants.

At Ninole and Waiakea, *Desmodium ovalifolium* was sown in the inter-row spaces. Because of heavy weed pressure and occasional herbicide applications, this took more than a year to attain reasonable cover. In some places at Ninole, good cover never occurred. At Waiakea, *D. ovalifolium* did not survive the low mowing (3 inches), combined with periodic herbicide drift, and was naturally substituted by *D. heterophyllum*.

Initially, all plantations were weeded mechanically, with a weedwacker, at six-week intervals. Within six months, however, weed populations built up to levels that required periodic herbicide applications. Glyphosate (Roundup, Monsanto) was used along the edge of the weed mat at Ninole and Waiakea and between plants at Poamoho, as well as for spot applications on Guinea grass (*Panicum maximum* Jacq.). Herbicide drift

occasionally affected palms and frequently affected the *Desmodium*. When the management interval was changed to once a month, rather than once in six weeks, weed populations were more easily managed mechanically and herbicide applications were reduced to spot applications for Guinea grass.

3.9. Fertilization

The fertilization levels were derived from recommendations made by Herrera (1989): 50 g N, 2 g P, 33 g K, 12 g Mg, and 71 g Ca per plant/year. Commercial preparations with approximately the same formula were used instead of preparing a specific mix.

At six, 10, and 14 months after field planting, all plants received 100 g of 18N-2.6P-10K, with minors, 6 to 9-month slow-release fertilizer (Osmocote, Sierra) as a dressing around their base. At 18 and 22 months, all plants received 100 g of 21N-3P-11.6K fast-release fertilizer (United Ag Products) as a dressing around their base. Starting at 24 months, 100 g of the 21-3-11.6 was applied at 2-month intervals, instead of 4 months, both because the plants were larger (two stems versus one stem) and because the 4-month interval had proved to be too long at Ninole and Waiakea, where rainfall is greater. The conclusion was based upon slight yellowing of the plants after 3 to 4 months.

At 12, 24 and 36 months after field planting, all plants received 100 g Ag-10 coarse-ground dolomite as a dressing. After the discontinuation of the slow-release with minors, no minors were applied.

3.10. Morphological Measurements

Morphological measurements were taken at approximately 6-month intervals during the course of the experiment to accompany growth. Most of the descriptors used were identified and tested by Clement (1986a, b).

The first set of measurements was taken on all plants of all ages and are presented below.

a. **Number of leaves** - The number of fully expanded green leaves in the crown of the stem to be harvested for its heart was counted. Mostly yellow leaves were ignored, as these were assumed to be photosynthetically inactive.

b. **Height** (cm) - The height from the soil surface beside the base of the stem to the fork between the first fully expanded leaf and the spear (or flag) leaf was measured. Measuring height in this way avoids the excessive variation in leaf expansion and curvature observed when trying to use the highest part of the first or second fully expanded leaf (Anderson 1983).

c. **Number of offshoots** - The pejibaye is a caespitose palm, meaning that it produces vegetative shoots from the base of the stem. These are called offshoots, suckers, or keikis (in Hawai'i). They are essential for guaranteeing the perenniality of a heart of palm plantation. The number of offshoots was counted.

The following set of measurements was taken on seedlings that only had bifid leaves. Although the pejibaye is a pinnate palm, early establishment-phase leaves are bifid. The separation of the leaflets, forming pinnate leaves, distinquishes the two parts of the establishment phase (see Chapter 4). This set of measurements was only taken during the

first six months to one year of field growth; it was not used on offshoots after harvest of the main stem.

a. **Length of the bifid leaf** (cm) - The length of one half of the bifid leaf was measured from the insertion of the first leaflet to the tip of the leaf on the side with the first inserted leaflet (Chapter 2, Figure 2.3).

b. **Width of the bifid leaf** (cm) - The width of one half of the bifid leaf was measured from the tip of the leaf rachis perpendicularly across the bifid blade on the side with the first inserted leaflet vein (Chapter 2, Figure 2.3).

c. **Spines on petiole/rachis** (ventral surface) - The number of spines in the 10 cm on either side of the first leaflet (3rd leaf) were estimated and the trees were classed using an ordinal scale of 0, 1-9 (1990). Changes in spininess of successive leaves during the maturation of the plant were also noted. Three classes were identified: 1 - constant spininess (including lack of spines, i.e., all spineless plants are classed as 1); 2 - slow or gradual change (decrease), so that all leaves in the crown still have some spines; 3 - fast change (decrease), where the oldest leaf is spiny and 3-5 leaves later they are spineless.

The third set of measurements was taken on all plants during the first year and a half in the field. It was discontinued after developing the allometric equations (see Chapter 4).

a. **Number of spear leaves** - The number of unopened leaves in the crown was counted. In practice, a true spear leaf (completely unopened) and a flag leaf (with leaflets starting to separate at the apical tip) frequently occur in the same plant. Both were considered spear leaves here. The longer spear leaf is generally the outer petiole sheath of the market quality palm heart (Clement et al. 1988)

b. **Sheath length** (cm) - The petiole in most palms is composed of a fibrous sheath that completely encloses the next inner leaf, and a section without the sheath immediately subtending the rachis. The sheath is differentiated from the petiole *sensu stricto* by the occurrence of loose fibers along its edge. These fibers originally enclosed completely the next newer leaf and were ripped apart by their growth, leaving a shaggy edge. The length of the petiole sheath was measured from the soil surface beside the stem to the apical end of the sheath.

c. **Petiole length** (cm) - The length of the petiole *sensu stricto* was measured from the end of the sheath to the insertion of the first leaflet, on the side with the first leaflet. The edge of the petiole is smooth, because it lacks the fibers of the sheath.

d. **Rachis length** (cm) - The length of the leaf rachis was measured from the insertion of the first leaflet to the apical tip of the rachis where the last leaflets form a bifid set (Chapter 2, Figure 2.2)

e. **Rachis width** (mm) - The width of the leaf rachis adjacent to the insertion of the first leaflet was measured with calipers (Chapter 2, Figure 2.2).

f. **Rachis thickness** (mm) - The maximum thickness of the leaf rachis adjacent to the insertion of the first leaflet was measured with calipers (Chapter 2, Figure 2.2).

The last two items were used by Corley et al. (1971) and Clement et al. (1990) to estimate leaf biomass in *E. guineensis* and pejibaye, respectively.

g. **Side with first leaflet** - The side of the rachis with the most basal leaflet, as observed from the position of the spear leaf looking out towards the leaf tip, was scored for left or right. This observation is directly related to the phyllotaxic direction of the plant.

h. **Number of leaflets** - The number of pinnate leaflets was counted on the side of the rachis with the first leaflet.

i. **Length of middle leaflet** (cm) - The number of pinnate leaflets counted in item n, divided by two, identified the middle leaflet. The length of this leaflet was measured from its insertion on the rachis to its tip.

j. **Maximum width of middle leaflet** (mm) - The position of the maximum width of the middle leaflet was determined approximately by eye and measured.

The final set of measurements were taken on the edible product at harvest.

a. **Heart weight** (g) - The sum of fresh weight of each of the one to six market quality sections extracted from immediately above the meristem. Each section is 9 cm long, which is the length of a section in a can. The determination of "market quality" was made with the tip of the thumb nail being pressed into the palmito section across the grain of the fibers. If the thumb nail did not enter the palmito tissue at any point along the length of the section, then the section was too fibrous to be considered market quality.

b. **Heart length** (cm) - The length of the market quality section. Because the market quality section is always divided into sections that are 9 cm long, it is a multiple of 9.

c. **Heart diameter** (mm) - The average diameter of the market quality sections, each section measured at its central point. The palm heart is generally elliptical in cross-section. Rather than take two measurements, only the narrower cross-section was used. The difference between the narrower and the thicker cross-section is generally only a couple of mm, so density estimates are only slightly off.

d. **Edible stem weight** (g) - The fresh weight of the stem immediately below the meristem that is tender enough to be eaten raw. The edible portion is distinquished by the ease with which it is cut across the fibers by a pocket knife.

e. **Edible leaf weight** (g) - The fresh weight of the edible leaf tissues that are not surrounded by petiole fibers. The edible portion extends from the last palm heart section to where the leaflets and rachis tissue become difficult to cut across the fibers with a pocket knife.

f. **Acridity** - A small section of petiole from the base of the first heart section was removed and tasted at the end of heart processing. An ordinal scale of 0-9 was planned, but Clement, the only regular taster, soon became relatively insensitive to acridity. Consequently, a 0, 1 scale was used, with 0 - undetectable by Clement, and 1 - detectable by Clement. This characterization is extremely inadequate but was the only one feasible during this stage of the USDA project. In the event that pejibaye becomes an important niche crop in Hawai'i, a taste panel, like the experimental panel organized by MS Catherine Cavaletto, will be required to adequately identify acrid plants.

g. **Total Soluble Solids** (%) - A small section containing the meristem was removed from the base of the first heart section and taken to the laboratory to measure TSS. The section was further reduced to contain approximately 2 mm below the meristem and 10 mm above it, with a diameter of 10-15 mm. This section was crushed quickly in a hand-held garlic press. The crushed tissue was reloaded into the garlic press and pressed gently to squeeze out 5-6 drops of liquid directly onto an Atago PR-1 refractometer. The reading was taken immediately, so as to avoid sedimentation of solids onto the optical reader.

3.11. Plant Morphology - Analysis

Lowess smoothing was used to show trends in scatter plots (Cleveland 1993). Lowess stands for Locally Weighted Scatterplot Smoothing (Minitab 1994). Because each plant was in a slightly different developmental phase at any given evaluation, height was used instead of age to show age-related trends.

Both the germination and the field experiments analyzed were replicated split-plots, with environmental effects (screen house vs. glasshouse, or plantation densities of 3333, 5000, 6666 plants/ha) as main plots and progenies as sub-plots. Transformations were used when appropriate, e.g., for frequencies of spiny plants the arcsine transformation was used (Little & Hills 1978).

3.12. Leaf Area and Biomass Allometry

A random sample of 25 pinnate leaves from plants of various sizes in the pinnate establishment sub-phase was harvested and the area measured to validate leaf area allometrics. On each leaf, rachis length, rachis width and thickness at the insertion of the first leaflet, side with first leaflet, number of leaflets, and middle leaflet length and maximum width on the side with the first leaflet were measured. The leaf area was then determined with a leaf area meter (Li-Cor Inc., LI-3000).

Leaves 2 and 3 were collected from a random sample of 10 plants that were harvested and measured as above to validate leaf biomass allometrics. The petiole, rachis and leaflets were separated and weighed. Leaf area was estimated from the equation

developed in Chapter 4. A sample was taken from each component, weighed, dried at 65°C for 5 days, and reweighed.

A random sample of 28 plants was harvested to develop whole plant leaf area and biomass allometrics. Each leaf was measured and weighed as above. Additionally, height, leaf number, spear leaf number and weight, and stem weight were measured. The spear leaves and stems were dried as above.

Analysis consisted of a series of simple and multiple regressions of the estimated variable (y) on the predictor variable(s) (x), with and without squaring and natural logarithm transformations, using Minitab 10.2. There are theoretical limitations to this method, since regression is correctly used to find the relationship between one normally distributed variable (y) and a 'mathematical' variable (x), where x can be time, regularly spaced treatments, etc (Causton & Venus 1981, Sokal & Rohlf 1981). Sokal & Rohlf (1981), however, consider regression acceptable if the objective is simply to develop a prediction equation.

The 'best' prediction equation within the series of equations was identified by its precision of estimation. Precision (or goodness of fit) of the equation was evaluated through the combination of the magnitude of the standard error of the estimate ($s_{y \cdot x}$), the coefficient of variation of the equation ($s_{y \cdot x}/\bar{Y}$), and the coefficient of determination (r^2) (Crow & Schlaegel 1988). In the case of log-log regressions, a generalized standard error of the estimate (s_e) was calculated based on equation predictions on the measured units, as suggested by Crow & Schlaegel (1988). The generalized $s_e = [\sum d^2/(n-2)]^{½}$, where $\sum d^2$ is the sum of squares due to deviation from regression (Little & Hills 1978). When

comparing non-transformed regressions with log transformed regressions, greater emphasis was placed on the standard errors and CV's.

When the power function was identified as a best model, a correction factor to counteract bias from fitting the model to log-transformed data (Baskerville 1972, Sprugel 1983) was tested for its effect on the s_e and CV. With the correction factor, 'a' = exp(a' + $s^2_{y\cdot x}/2$), where *a'* is the *a* obtained from the log-log regression output, and $s_{y\cdot x}$ is the standard error of the estimate obtained from the same output. If the s_e and CV were reduced by the correction factor, it was used; where no reduction was observed, it was not used.

The identity of the slopes of the bifid eophyll whole plant leaf area and biomass regressions versus the slopes of the pinnate-leaved whole plant leaf area and biomass regressions was tested by the t-test:

$$t = (b_1 - b_2)/s_{b1-b2}$$

where $s_{b1-b2} = (s^2_{b1} + s^2_{b2})^{1/2}$ and the degrees of freedom for the test are $n_1 + n_2 - 4$ (Glantz & Slinker 1990).

3.13. Growth Analysis

All plants at Ninole, Poamoho, and Waiakea were measured several times and at harvest. At Ninole, there were six measurement dates, while at Poamoho and Waiakea there were three dates.

The allometric equations developed in Chapter 4 were used to estimate whole plant leaf area and biomass. These estimated values were used in the growth analysis formulae.

In the chapters on results, especially Chapters 5 and 6, the growth parameters are all the result of estimation, although the qualification 'estimated' is not used. Additionally, because there were errors associated with using the allometric equations, as well as occasional experimental error and wide variation in plant size at any given time, units of variation are not given with the growth parameters. The reason is that it is not possible to separate the sources of variation from each other.

The following growth analysis formula were used:

$$CGR = (W_2 - W_1)/(t_2 - t_1);$$

$$\text{mean } RGR = (\log_e W_2 - \log_e W_1) / (t_2 - t_1);$$

$$\text{mean } E_A = [(W_2 - W_1)/(L_{A2} - L_{A1})]*[(\log_e L_{A2} - \log_e L_{A1})/(t_2 - t_1)];$$

$$\text{instantaneous } LAR = L_A/W;$$

$$\text{mean } LAR = [(L_{A1} / W_1) + (L_{A2} / W_2)] / 2,$$

where W is whole plant biomass (kg, dry weight basis), L_A is whole plant leaf area (m^2), and t is time at a given day after planting, and 1 and 2 refer to an two measurements dates.

It is important to note that LAR does not include the full year's leaf production, only that at the moment of each evaluation. Leaf duration appeared to be less than one year, because a plant should produce about one leaf per month (Mora Urpí 1984), but there were seldom more than 6-8 leaves at any one time.

3.14. Quantitative Genetic Analysis

Phenotypic and additive genetic variances were estimated from the components of variance in each experimental design (see section 3.6). Only individual mean heritabilities were calculated, since future selection in this population will be based upon individuals, rather than progenies.

The progenies used in this project are half-sibs. Therefore, the additive genetic variance is 4 times the covariance of the half-sibs (Falconer 1981, Nyquist 1991). This covariance is represented by the progeny variance, after removal of the appropriate amounts of progeny x environment interaction variances. The more complex the experiment, the better the estimate of the additive genetic variance (Simmonds 1979). Thus, at the Ninole site for example, the best estimate was obtained from the full density x progeny trial. Each trial and site had a different set of progenies (Table 3.2.).

Table 3.2. Pejibaye Benjamin Constant progenies present in each trial at each site and the number of plants in the trial.

Site (density)	Progenies	Number of Plants
Ninole (density trial)	0, 1, 2, 3, 5, 8, 9	567
Ninole (3333)	0, 1, 2, 3, 5, 8, 9	189
Ninole (5000)	0, 1, 2, 3, 5, 6, 7, 8, 9	243
Ninole (6666)	0, 1, 2, 3, 5, 6, 8, 9	216
Poamoho (5000)	1, 2, 3, 5, 6, 8, 9	183
Waiakea (5000)	0, 1, 2, 3, 6, 8	162
N & P (5000)	1, 2, 3, 5, 6, 8, 9	372
N & W (5000)	0, 1, 2, 3, 6, 8	324
N & P & W (5000)	1, 2, 3, 6, 8	405

Theoretically, a better estimate is obtained from a multi-location, multi-year trial (Nyquist 1991), but this was not true for this project because of extremely strong location effects and location x progeny interactions. Because these strong interactions are partially due to inadequate management (lack of irrigation at the beginning of the Poamoho trial; too much time between fertilizer applications at Ninole and Waiakea during the first two years) and periodic drought at Waiakea, genotype x environment interactions were not studied in detail but are presented graphically for a few traits.

The quantitative results are presented as the magnitude of the phenotypic variance (V_p), the coefficient of phenotypic variation ($CV = V_p^{1/2} / mean * 100$ (Brewbaker 1994)), and the narrow-sense heritability (h^2), always assuming that these progenies are from an outcrossing panmictic population. The additive genetic variance, although frequently mentioned, was not presented in the tables. It is calculated as: $V_p * h^2$. Since there were strong indications of inbreeding, however, the additive genetic variance must be multiplied by 1-F (Falconer 1981).

3.15. Enzyme Electrophoresis

Approximately thirty plants selected randomly from each progeny were sampled for electrophoretic analysis. Enzymes were obtained either from leaf tissue (Rojas Vargas 1993) or the meristem juice used to determine total soluble solids (see section 3.10). An 8-mm-diameter leaflet disk was ground in, or six drops of meristem juice were mixed with, three drops of fresh, chilled extraction buffer (Wendel & Weeden 1989, Lebot & Aradhya 1991), to which was added β-mercaptoethanol immediately before use. The

resulting slurry was absorbed on 2- x 10-mm chromatographic paper wicks, blotted to remove excess slurry, and either used immediately or frozen for later use. When the electrophoresis was planned for the next day, the wicks were frozen at -20°C; when planned for more than a day later, they were frozen at -60°C.

Five gel/tray buffer combinations were evaluated with leaf tissue extract: tris-citrate, pH 7.5; histidine-citrate, pH 6.5; morpholine-citrate, pH 6.0; sodium-borate, pH 8.0/tris-citrate, pH 8.6; tris-borate-EDTA, pH 8.6 (Wendel & Weeden 1989). Fresh or frozen wicks were loaded directly into starch gels previously prepared and cooled to 4°C. Electrophoresis was conducted at 4°C for 5.5 hours at the indicated amperage and voltage for each system. Six enzyme systems (GPI, MDH, PGM, SKDH, TPI, UGPP) were used as standards for the buffer evaluations.

After selection of a standard gel/tray buffer system, the following enzyme systems were evaluated for activity, resolution, probable number of loci and ease of probable genetic interpretation with both tissue extracts: 1) acid phosphatase (ACP: EC 3.1.3.2); 2) aconitate hydratase (ACO: EC 4213); 3) adenylate kinase (ADK: EC 2.7.4.3); 4) alcohol dehydrgenase (ADH: EC 1.1.1.1); 5) alkaline phosphatase (ALP: EC 3.1.3.1); 6) arginine aminopeptidase (AMP: EC 3.4._._); 7) aspartate aminotransferase (AAT: EC 2.6.1.1) [= glutamate oxaloacetate transaminase (GOT)]; 8) catalase (CAT: EC1.11.1.6); 9) diaphorase (DIA: EC 1.6.4.3); 10) endopeptidase (ENP: EC 3.4._._); 11) esterase (EST: EC 3.1.1.1); 12) formate dehydrogenase (FDH: EC 1.2.1.2); 13) fructose-bisphosphatase (FBP: EC 3.1.3.11); 14) glucose-6-phosphate dehydrogenase (G6PDH: EC 1.1.1.49); 15) glucose-6-phosphate isomerase (GPI: EC 5.3.1.9); 16) glutamate

dehydrogenase (GDH: EC 1.4.1.2); 17) glyceraldehyde-3-phosphate dehydrogenase (G3PDH: EC 1.2.1.12); 18) glycerate-2-dehydrogenase (G2DH: EC 1.1.1.29); 19) hexokinase (HK: EC 2.7.1.1); 20) isocitrate dehydrogenase (IDH: EC 1.1.1.42); 21) leucine aminopeptidase (LAP: EC 3.4.11.1); 22) malate dehydrogenase (MDH: EC 1.1.1.37); 23) malic enzyme (ME: EC 1.1.1.40); 24) mannose-6-phosphate isomerase (MPI: EC 5.3.1.8); 25) menadione reductase (MNR: EC 1.6.5.2); 26) peroxidase (PRX: EC 1.11.1.7); 27) phosphoglucomutase (PGM: EC 5.4.2.2); 28) phosphoglutonate dehydrogenase (PGD: EC 1.1.1.44); 29) shikimate dehydrogenase (SKDH: EC 1.1.1.25); 30) sorbitol dehydrogenase (SDH: EC 1.1.1.14); 31) superoxide dismutase (SOD: EC 1.15.1.1); 32) triose-phosphate isomerase (TPI: EC 5.3.1.1); 33) uridine diphosphoglucose phyrophosphorylase (UGPP: EC 2.7.7.9). EC codes are from Dixon & Webb (1964). Stain recipes were obtained from Wendel & Weeden (1989) and Shaw & Prasad (1970).

Stained gels were evaluated and probable interpretations coded alphabetically. The BioSys 1.7 population genetics program (Swofford & Selander 1989) was used for analysis of within- and among-progeny allozyme variation. Allelic frequencies and observed heterozygosity for each polymorphic locus, deviations from Hardy-Weinberg expectations at each locus and over all loci, Wright's fixation index for each locus in each progeny, Wright's F statistics, and Nei's unbiased genetic identities and distances were estimated, and cluster analysis was performed on the genetic identity matrix with the UPGMA algorithm to portray progeny relationships in a phenogram. Individual mean heterozygosities were computed from the allozyme genotypes observed and were used in

calculating the correlation coefficients with earliness, RGR, E_A, LAR, spines, offshoot number, heart and total product weight.

Chapter 4. Morphology and Allometry of Juvenile Plants

4.1. Introduction

In palms there are two phases of juvenility (seedling and establishment) and one early mature phase (adult vegetative), which are typified by purely vegetative growth (Tomlinson 1990). The economic production of heart of palm is concerned only with these three phases. Each phase has a distinctive morphology (Tomlinson 1990) and may have phase-specific allometric relationships among plant parts, as occurs in other species (Causton & Venus 1981). These relationships can be used to estimate the dimensions of other parts. The objectives of this chapter are (1) to describe the morphology of the vegetative growth phases and (2) to describe the allometric relationships that are useful for estimating individual and whole plant leaf area and biomass in the establishment phase.

A good morphological description of mature pejibaye exists (Chapter 2), but the seedling and later juvenile stages are poorly described. This description is important in the study of an economic palm because relationships between juvenile morphology or growth and other economically important traits often exist (Tomlinson 1990). For example, there are often correlations between growth rates of juvenile and mature plants, and between juvenile growth rate and early maturity. The importance of these relationships accounts for the existence of good descriptions of the seedling and juvenile growth phases of coconut (*Cocos nucifera* L.) (Child 1974) and African oil palm (*Elaeis guineensis* Jacq.) (Corley 1976, Hartley 1977).

There are two ways of conceptualizing a palm such as coconut, African oil palm, or a single stem of pejibaye: 1) as a collection of organs that can be studied individually and put together into a unit; or 2) as an integrated whole that is studied as a unit. Both are valid and provide insights into the biology of the plant.

In African oil palm, Hardon, Corley and their collaborators took the first approach in developing methods to estimate whole plant leaf area and biomass from other plant dimensions. Hardon et al. (1969) estimated the area of single leaves from leaflet number and the dimensions of a sample of six leaflets. Corley & Hardon (unpublished, cited by Corley (1976)) estimated seedling leaf area from leaf length and width. Corley et al. (1971) estimated individual leaf biomass from petiole dimensions and stem biomass from its dimensions and known or assumed age. Whole plant leaf area was then obtained from mean leaf area (n = 2) x leaf number, and whole plant biomass from mean leaf weight x leaf number plus stem weight. The African oil palm researchers developed single estimators for the entire species, rather than developing distinct estimators for each population or progeny. This approach is excellent for detecting differences among plants due to genetic or environmental factors, because each plant is treated as an individual even though the estimators are general.

In pejibaye, a similar approach was followed. A detailed morphological description of the mature leaf (Clement & Mora Urpí 1983) was followed by adaptation of African oil palm methods to pejibaye: for estimation of individual leaf area (Clement et al. 1985), for estimation of seedling leaf area (Clement & Habte 1994), and for estimation of leaf biomass (Clement et al. 1990). In the seedling and establishment phases, whole plant

biomass can be estimated from these data because no stem is present. Martel & Clement (1986) found that three populations of pejibaye from different landraces had similar leaf area equations, while Clement et al. (1990) developed their leaf biomass equation for three populations, representing different landraces. In each case, the estimation equations were compared and found not to differ among populations (see Chapter 2). This suggests that, as in African oil palm, there are good estimation equations for the whole species.

The "pipe model" and the high correlation between the growth of one part of an organism and growth of the whole are the basis for the integrated approach for developing whole plant estimators. Tomlinson (1990) shows that palms in general are good examples of the "pipe model" theory of tree form (Shinozaki et al. 1964), which holds that a "unit pipe" of conducting and support tissue supports a "unit of crown," both mechanically and hydraulically. This model provides the theoretical basis for the allometric relationships between stem diameter and whole plant leaf area widely used in forestry, e.g., Waring (1983). Foresters use other allometric relationships to estimate bole or whole plant biomass from tree height and stem diameter (Waring 1983, Crow & Schlaegel 1988). I have been unable to find reference to the use of this integrated approach in African oil palm.

Szott et al. (1993) pioneered use of the integrated approach in pejibaye. Using germplasm of the Yurimaguas population of the Pampa Hermosa landrace, these workers found plant height, stem diameter and leaf number to be good estimators ($r^2 > 90\%$) of above ground whole plant biomass and leaf biomass (see Chapter 2). They also found that the allometric relationships of stemmed plants less than 4.2 m tall, which

corresponds roughly to the adult vegetative phase, and plants more than 4.2 m tall, which corresponds roughly to the adult reproductive phase, were different. They did not test the significance of the difference between the allometric equations for the two phases, however.

In this chapter, I describe the morphology of the seedling, establishment and early adult vegetative phases of pejibaye. The establishment phase can be divided into two sub-phases based on leaf form: the bifid eophyll phase, where leaves are entire, and the pinnate phase, where leaves are fully pinnate. The development of branches, called suckers, off-shoots, or keikis (in Hawai'i) is given special attention, as they are essential for maintaining the longevity of a plantation. The allometric relationships among various plant dimensions were examined to validate or expand earlier work on estimators of individual and whole plant leaf area and biomass and define equations for use in Hawai'i. The existence of distinct allometrics in the two establishment sub-phases was tested.

4.2. Morphology of the Seedling Phase

After germination, the first structure to emerge above the substrate is a 1-2 cm long sheath, that looks like a small green spike. This sheath may bear spines. The radicle may or may not appear by the time the first sheath is extended. The second visible structure is also a sheath, that can be up to 8 cm in length. If the plant has the gene(s) for petiole spines, spines will almost always be present on this sheath. (The gene(s?) for petiole spines is expressed on the sheath, petiole and rachis; petiole spines is a short-hand term for this gene.) The radicle is always present by the time the second sheath has expanded

and elongates to 4-6 cm. Adventitious roots appear from the hypocotyl during radicle growth.

The third aerial structure is the first bifid eophyll, which ranges in length from 4 to 19 cm; very rarely a third sheath is produced. A bifid eophyll is a leaf with two lobes, with each lobe being composed of several to numerous fused leaflets (Chapter 2, Figure 2.3). The bifid eophyll has a distinct sheath, short petiole, and short rachis supporting the fused leaflets. The second bifid eophyll is larger than the first, ranging in length from 6 to 27 cm. During the next two to three months of growth, the four to five leaves produced are bifid eophylls, each larger than the previous one (see Chapter 5 for two growth measurements at transplant).

Spines, when present, are found on either the petiole, the leaflet mid-ribs or the edges of the leaflets, or any combination of these. Significant differences in spininess among the progenies were evident at the first and second bifid eophyll stage (Table 4.1).

4.3. The Establishment Phase

The establishment phase extends from endosperm depletion to the appearance of the stem (Tomlinson 1990). Since endosperm reserves can not be observed directly, the transition to the establishment phase is somewhat arbitrary. I have assumed that endosperm reserves are depleted by the full expansion of the fifth bifid eophyll. Pejibaye's establishment phase can be divided into two sub-phases: the bifid eophyll sub-phase, with entire leaves; and the pinnate-leaved sub-phase, with partially to fully pinnate leaves.

Table 4.1. Frequencies of spiny pejibaye plants by progeny at time of transplanting to the nursery (i.e., with one or two bifid eophylls; sheathed plants not included).

Progeny	n	Petiole	Leaf Mid-rib	Leaflet Edge
B-0	102	0	0.588	0.100
B-1	153	0.153	0.617	0.256
B-2	158	0.236	0.686	0.320
B-3	129	0.244	0.696	0.292
B-5	105	0.324	0.685	0.370
B-6	140	0.285	0.781	0.435
B-7	34	0.187	0.430	0.145
B-8	144	0.304	0.791	0.465
B-9	108	0.315	0.705	0.318
mean		0.228	0.664	0.300
S.D.		0.097	0.103	0.114

4.3.1. Morphology of the Bifid Eophyll Sub-phase

During this phase, all leaves produced are bifid eophylls, each larger than the next. At the beginning of this sub-phase, the plants have 4-5 leaves and are 2-5 cm tall. The dimensions of the larger leaf lobe of the newest leaf range from 9 to 20 cm in length by 2 to 5 cm in width. By the end of this sub-phase, the plants have 6-10 leaves and are 20-30 cm tall, rarely 40 cm. The larger leaf lobe of the newest leaf ranges from 30 to 50 cm in length by 10 to 20 cm in width. Over the range of heights between 20 and 30 cm, the transition from a bifid eophyll to a pinnate leaf occurs (Figure 4.1). The leaf axis lengthens significantly during this sub-phase, with the petiole lengthening proportionately more than the rachis or sheath, probably as an adaptation to avoid shading of the older leaves by the newer leaves (Tomlinson 1990).

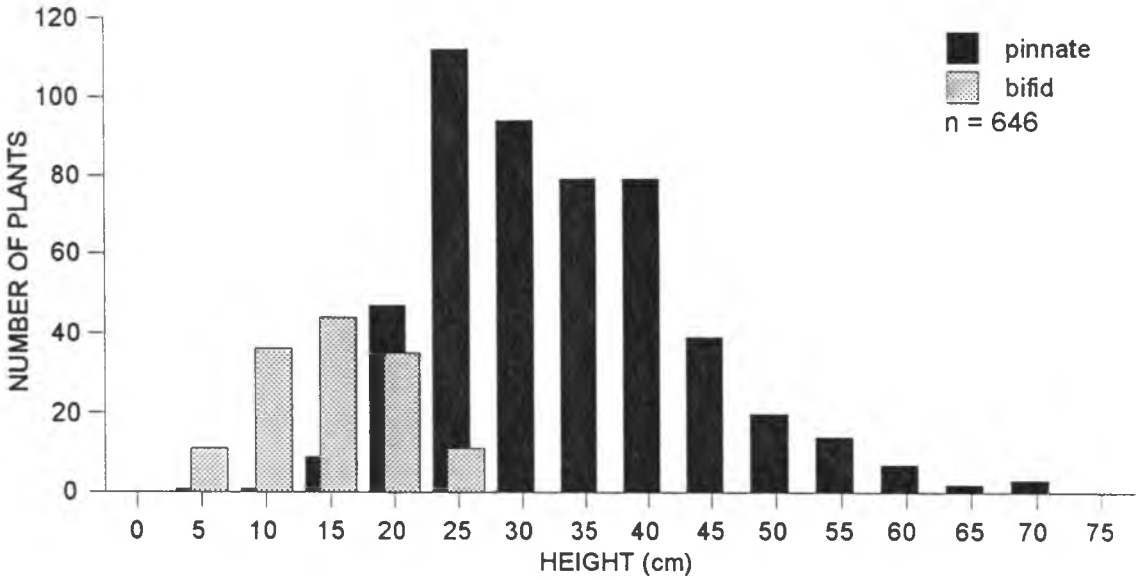

Figure 4.1. Frequency of bifid eophyll and pinnate sub-phase pejibaye palms by height class at Ninole after 293 days in the field.

The morphology of the bifid eophyll changes over time. During and immediately after the seedling phase, the eophyll blade is entire and slightly and regularly corrugated. The corrugated effect is due to the reduplicate morphology of the leaflets, where the leaflet mid-rib can be viewed (in cross-section) as the apex of a triangle, the two sides adjacent to the apex are the leaflet lamina, and the opposite side is open. Near the end of this sub-phase, the eophyll blade becomes irregularly corrugated as leaflets are pulled out of the rachis plane by the developing pulvini (swellings at the base of each leaflet) and the elongating rachis. Finally, at the end of this sub-phase, splits appear between some adjacent leaflets and the leaf starts to become pinnate.

In the bifid eophyll sub-phase, petiole spininess shows the beginnings of three tendencies: 1) constant - the spininess or spinelessness does not change from the juvenile

to the mature plant; 2) slow change - the spininess evident in the seedling phase slowly becomes less evident, with each succeeding leaf having slightly fewer spines; or 3) rapid change - the spininess evident in the seedling phase rapidly becomes less evident until there are no spines on the petiole. Change in spininess during development was also observed in Yurimaguas germplasm (Chávez Flores et al. 1990).

The frequency of constantly spineless plants in this population was 74.5%, while that of constantly spiny plants was 12%. The frequency of slow change spiny plants was 11%, while that of rapid change spiny plants was 2.5%. The latter two tendencies suggest that there is a developmental system where spines are expressed in the juvenile phase, when spines would provide the greatest protection to the leaf, but are suppressed in the mature phase, when the leaves have grown beyond the reach of large herbivores. J. Mora Urpí (pers. comm.) hypothesized that a given spine trait, e.g., petiole spines, is a semi-quantitative trait, with a major gene and several modifiers. The change in expression of spines during development is clearly a modification of the expression of the underlying major gene and supports his hypothesis.

The third tendency results in adult vegetative and reproductive phase plants that are spineless on the petiole. This apparent spinelessness can confuse the characterization of spininess in pejibaye. Consequently, for correct characterization this trait should be evaluated in juvenile plants, as done with seedlings by Chávez Flores et al. (1990) and with bifid eophyll and early pinnate sub-phase plants as described in Chapter 6.

4.3.2. Morphology of the Pinnate Sub-phase

During this phase, all leaves produced are pinnate, each larger than the next. At the beginning of this sub-phase, the plants have 6-10 leaves and are 20-30 cm tall, rarely 40 cm. By the end of this sub-phase, the plants have 6-12 leaves and are 110-120 cm tall. The late pinnate sub-phase leaf has 160-220 leaflets. The middle leaflet on the side with more leaflets ranges from 40 to 75 cm in length by 20 to 45 mm in width. The petiole shortens progressively over this period. During this period the rachis lengthens rapidly.

Tomlinson (1990) stated that the crown attains its mature leaf number and the leaves attain their mature size by the end of the establishment phase. This was not true for pejibaye in this project in Hawai'i. Leaf number at the end of the establishment phase in Hawai'i rarely exceeded 10 (Figure 4.2) and averaged only 6.7. Between the first and second evaluation dates there was a decrease in leaf number, the exact opposite of what was expected. In African oil palm there is a continual increase in leaf number with age from seedling to adult reproductive phase at fruiting density (Corley & Gray 1976a). The Lowess curve at 292 days after planting (Figure 4.2) is close to the curve presented by African oil palm, whereas the Lowess curve at 486 days does not continue to rise. This is not a density effect, as there were no significant differences between the densities at Ninole at that time. *In situ*, mature plants of the Benjamin Constant population averaged 17 leaves (Clement et al. 1988). In São Paulo, Brazil, germplasm from the Yurimaguas population averaged 6 leaves at one year and 8 at two (Bovi et al. 1987). During a visit to Manaus in 1995, I found an average of 10 leaves on two year old Pará landrace plants.

Leaf rachis length at harvest, which is the early adult vegetative phase, averaged 160 cm, and never exceeded 210 cm. *In situ*, leaf rachis length of mature Benjamin Constant plants averaged 280 cm (Clement et al. 1988).

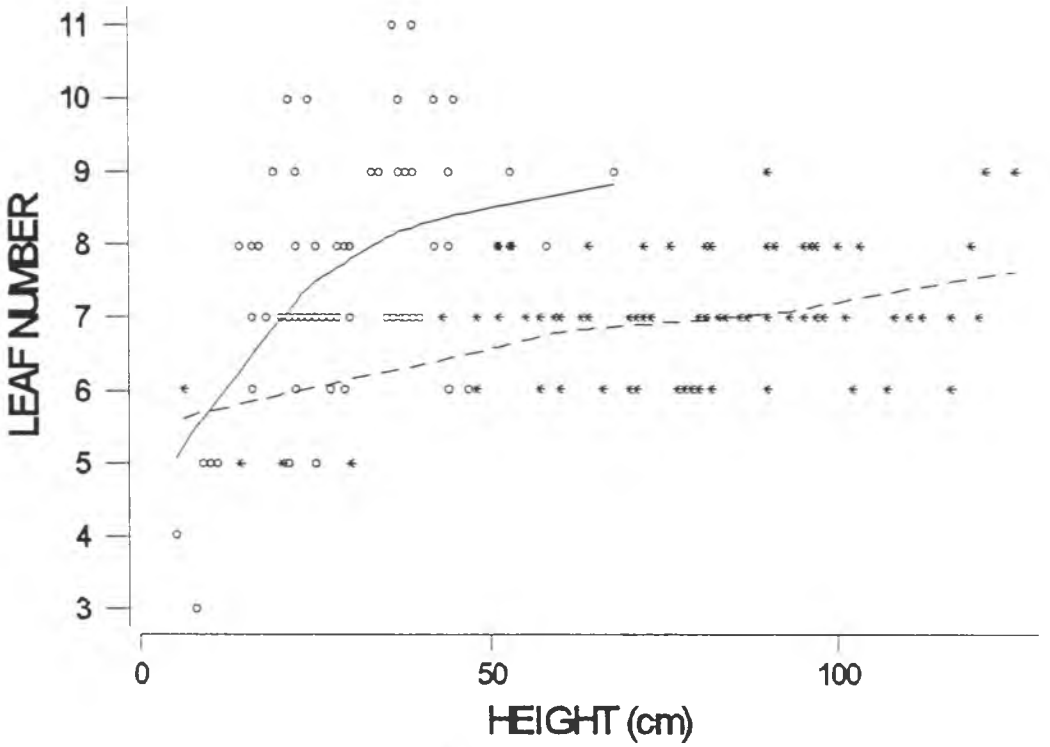

Figure 4.2. Pejibaye leaf number as a function of plant height at two dates at Ninole. Only the three center row plants of each plot in the 5000 plants/ha density are presented. Symbols: o - at 292 days after planting (dap); * - at 486 dap; – Lowess curve at 292 dap; -- Lowess curve at 486 dap.

It is not clear why pejibaye in Hawai'i should have fewer leaves than elsewhere. São Paulo is a similar distance from the equator and plants have fewer leaves than in Manaus, which is at the equator, but more than in Hawai'i. Thus, a physiological response to a latitude-related variable may be a factor, but is probably not sufficient by itself to explain this low leaf number. The micro-nutrient status of the degraded soils at Ninole and

Poamoho may be a factor, since leaf number increased rapidly during the first year in the field (to an average of 7.6), when they were receiving micro-nutrients with the slow-release fertilizer, and then leaf number decreased when they received only NPK (to an average of 7.0 at a year and a half, and 6.5 at harvest) (Figure 4.2). Since leaf number is an important growth parameter, this question deserves further consideration in Hawai'i.

Offshoots are extremely important in pejibaye for heart of palm production, because they allow for long-term production without need for replanting. Offshoots start appearing in the bifid eophyll sub-phase and become continually more numerous (Figure 4.3) and larger during the pinnate sub-phase. The maximum number observed was 18, at Waiakea.

Occasional plants never produced offshoots. The mean number of offshoots increased with plant age (Table 4.2) and size (Figure 4.3) and is an important progeny characteristic for any improvement program. The full potential of a given plant is only evident at the end of the establishment phase. Theoretically, there can be as many offshoots as leaves produced during the establishment phase (Tomlinson 1990).

No study of offshoot biomass accumulation was made, a shortcoming of this research. Offshoots are additional sinks requiring energy and nutrients that come initially from the main stem and may compete with the main stem for resources. Different progenies appeared to partition energy differently to their offshoots. Some progenies, e.g., B-1, partitioned resources early to offshoot number (Table 4.2). This did not appear to slow growth of the principal stem, as B-1 was one of the faster growing progenies. The offshoots of B-1 did not become large quickly, however, suggesting that less was

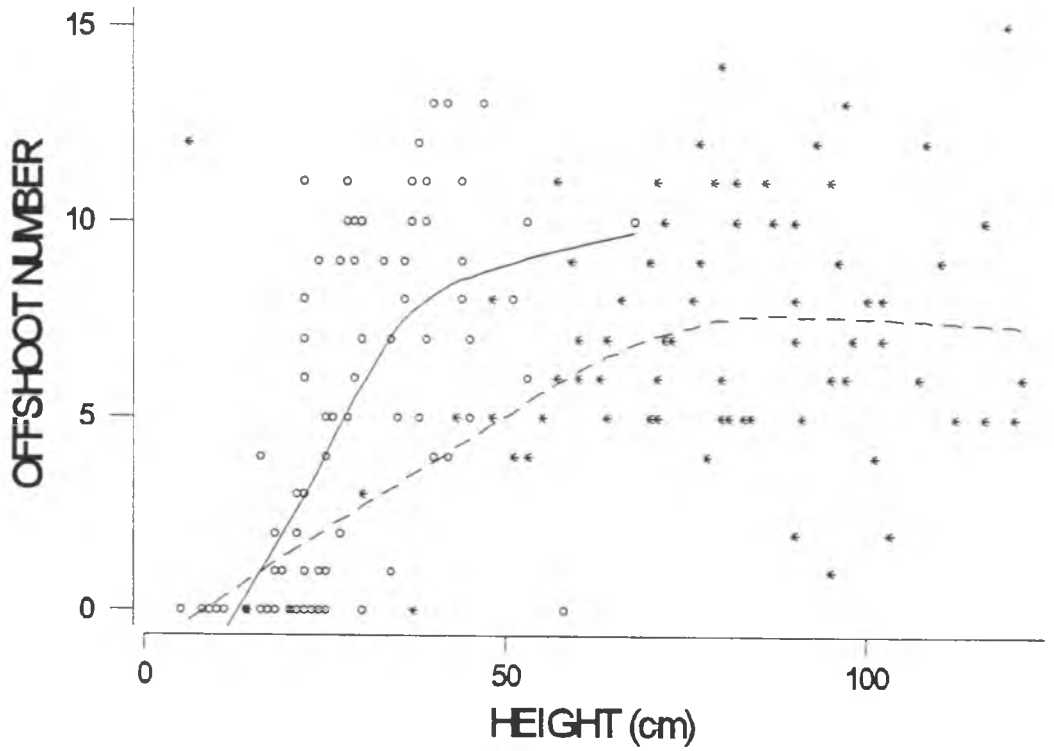

Figure 4.3. Pejibaye offshoot number as a function of plant height at two dates at Ninole. Only the three center row plants of each plot in the 5000 plants/ha density are presented. Symbols: o - at 292 days after planting (dap); * - at 486 dap; – Lowess curve at 292 dap; - - Lowess curve at 486 dap.

Table 4.2. Mean offshoot number of each pejibaye Benjamin Constant progeny at Ninole at three evaluation dates (expressed as days after field planting - DAP).

	at 175 DAP		at 293 DAP		at 488 DAP	
Progeny	mean±SD	min-max	mean±SD	min-max	mean±SD	min-max
B-0	1.9±2.0	0-7	6.3±2.5	0-11	6.8±2.2	1-13
B-1	3.1±2.1	0-8	8.3±2.9	0-13	9.1±2.4	4-15
B-2	1.3±2.1	0-9	3.8±3.8	0-13	6.6±3.1	0-13
B-3	0.2±0.6	0-4	3.1±2.9	0-11	5.5±2.8	0-11
B-5	0.9±2.0	0-8	3.7±4.1	0-13	6.5±3.9	0-15
B-8	0.1±0.4	0-3	3.1±2.6	0-9	5.5±2.3	0-11
B-9	1.6±1.7	0-6	5.1±3.2	0-10	6.4±2.6	0-12
mean	1.3±2.0	0-9	4.8±3.7	0-13	6.6±3.0	0-15

partitioned from the main stem to the offshoots after a certain amount of growth had occurred. Other progenies, e.g., B-3, partitioned fewer resources to offshoot growth at all times, although this did not appear to result in faster main stem growth, as the growth rate of B-3 was only average. Some progenies, e.g., B-9, partitioned significant resources to offshoots, as indicated by offshoot size, rather than number. Progeny B-9 was vigorous and the partitioning of energy between the main stem and the offshoots had no apparent effect on growth of the main stem. More attention is required to this subject in order to better design the offshoot component of the pejibaye ideotype for a heart of palm improvement program.

4.3.3. Morphology of the Regrowth Establishment Phase

After harvest of the first stem, the offshoots become independent quickly as the stem dies and rots. At harvest of the main stem, their development is slowed due to sun-scorch after removal of the shade provided by the main stem. Also, until harvest the offshoots are assumed to have received varying amounts of resources from the principal stem. This resource supplement is eliminated at harvest, just when the offshoots require more energy to withstand and recover from sun-scorch. As a consequence, growth of the offshoots may cease for a month or more, depending on the size of the offshoot at harvest. Even very small offshoots, e.g., 2 cm tall, generally survive this trauma, however.

Once growth starts again, it is much more rapid than during the first establishment phase (see Chapter 5). Morphology, however, is almost identical. There are two apparent differences, one minor, the other important. The minor difference is with respect to

crown shaft girth. After growth starts again, the crown shaft appears to be thicker than a seed-derived plant of similar height. Leaf biomass and area, however, do not appear to be affected, as regrowth leaves fit smoothly into the data sets used to develop leaf area and biomass prediction equations.

The important difference is off-shoot number. At the second harvest, the mean(±SD) offshoot number was 2±1.3, with zero being more common than at first harvest and only a single plant with a maximum of 10 offshoots. There are at least two possible explanations for this very low number of offshoots.

The first explanation is the way this plantation was managed. The full complement of offshoots was allowed to develop until harvest of the principal stem. An average plant had six or more offshoots, each with three to six leaves. This high number of offshoots and leaves can be equated with intense intra-plant competition. This competition, in turn, may have decreased partitioning of resources to the offshoot's own offshoot buds, causing their abortion or severe atrophy. On a poor Oxisol in Brazil, an increase in planting density from 4444 to 6666 to 10,000 plants/ha reduced mean offshoot number from 6.8 to 5.7 to 4.6 per plant (Moreira Gomes et al. 1988). The effective level of competition at the base of a stem with its maximum number of offshoots was probably higher than with 10,000 plants/ha. This type of density effect is conceptually similar to the 'self-thinning rule' in forestry and tillering grasses (Firbank & Watkinson 1990), which lends support to the explanation given here.

In commercial plantations in Costa Rica, the number of offshoots is managed continually, so that there are seldom more than necessary to maintain the clump.

Although plantation density is extremely high, 5,000 clumps/ha with 3-4 stems each, plants are harvested earlier (mean height about 1 m). An early harvest probably results in less competition than the later harvest used in this project (mean height of 1.37 m). Consequently, offshoot buds probably suffer less atrophy or abortion from competition in Costa Rican commercial plantations, even though densities are high. With native Costa Rican germplasm, which produces more abundant offshoots than the Amazonia germplasm used in this project, there is never any problem with having enough offshoots to sustain the clump.

The second explanation is the time between harvests. Between germination and the first harvest, plants averaged more than 24 months of growth, some as much as 36 months. Between the first harvest and the second, the regrowth cycle averaged about 12 months. There was much less time for offshoot buds to differentiate and start growth between harvests. On the most precocious second-harvest plants, most offshoots were less than 5 cm tall. There also may be differences in partitioning of resources to offshoots: first-harvest cycle plants may partition more resources to offshoots than second-harvest cycle plants.

On balance, and given the Costa Rican experience, I think that the excessive within clump competition allowed during the first harvest cycle probably caused the majority of the reduction in offshoot number at the second harvest observed here. The shorter period between harvests is probably a minor contributor to reduced number. This hypothesis can easily be tested during the coming years, by continuing to observe offshoot number on stems that are managed according to commercial practice. If the third and successive

harvests have more abundant offshoots than the second harvest, there is no need for further worry. If, however, offshoot number continues low or even decreases, the longevity of the plantation will be compromised.

In 1994, Mario Pinedo (personal communication) transmitted an observation from Costa Rica that some spineless germplasm produced fewer offshoots at each successive harvest until the clump died out. Unfortunately, attempts to obtain more detailed information have been unsuccessful. Given the variation observed in mean offshoot number per progeny (Table 4.2) and apparent differences in partitioning of resources by the principal stem to the offshoots (see above), it would not be a complete surprise if some plants, perhaps even some progenies, followed the trend observed in Costa Rica.

4.4. Morphology of the Adult Vegetative Phase

The transition from the establishment to the adult vegetative phase is defined by the appearance of the stem above ground level (Tomlinson 1990). The combination of a visible stem and a plant height of at least 130 cm was used to identify plants for harvest in this project. In general, the early adult vegetative phase plant looks morphologically like a late pinnate sub-phase plant except for the stem.

The first sign of the stem is a swelling of a leaf insertion on the stem just above ground level. This swelling forms a ring around 50-75% of the stem circumference. When that leaf senesces and falls, the stem is visible. At Ninole, stem diameter at this time ranged from 10.5 to 17.2 cm, with a mean of 13 cm. This mean is much smaller than the 19 cm observed *in situ* for the same germplasm (Clement et al. 1988), but the

observation of Szott et al. (1993) that girth continues to expand during the adult vegetative phase in Yurimaguas may explain part of this difference.

4.5. Allometric Relationships

In this section, only those allometric relationships that contribute to developing predictors of individual leaf or whole plant leaf area and biomass are examined. The correlation matrices, however, may indicate other relationships that are worth pursuing for other reasons, since these correlations are population specific and are themselves good traits for population discrimination (Sokal & Rohlf 1981). Since individual plants at some stage may contain both bifid eophylls and pinnate leaves, both are examined before looking at the whole plant.

4.5.1. The Bifid Eophyll

The dimensions of a random sample of bifid eophylls and the Pearson correlation coefficients between each pair of variables are in Appendix B. Scatter plots of rectangular leaf area versus measured leaf area, and versus leaf biomass (Figure 4.4), confirm the strong correlation between these pairs of variables and suggest their use for estimating leaf area and leaf biomass, respectively. The extra scatter in the leaf biomass plot is probably due to using some leaves with immature sheaths, which have less biomass per area than a more mature leaf.

The very high correlation between the rectangular leaf area (blade length x width) and measured leaf area suggested that this pair of variables would be sufficient to

develop a prediction equation, as found in African oil palm by Corley (1976) and Clement & Habte (1994). The Minitab regression routine detected possible curvature in the rectangular leaf area (Table 4.3, model 1), due to a lack of residuals in the lower left and right corners of the residuals versus predicted values plot (graph not shown). Both the square of the rectangular leaf area and the transformed variables were tested to determine if these transformations improved the goodness of fit. Of these various attempts, only the power function is shown (Table 4.3, model 2). This equation increased s, suggesting that neither squaring nor log-transformation could solve this slight lack of fit.

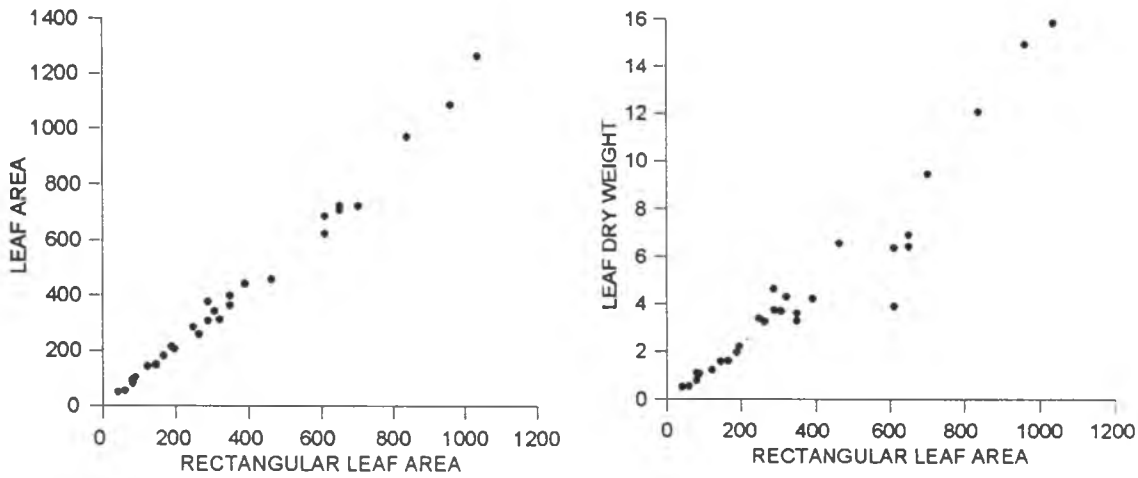

Figure 4.4. Scatter plots of rectangular leaf area (= blade length (cm) x blade width (cm)) versus leaf area (cm^2) and leaf dry weight (g) for the 28 pejibaye bifid eophylls used to develop the leaf area allometric equations.

The addition of the rachis cross-section at the insertion of the first leaflet decreased the magnitude of s (Table 4.3, model 3). Also, all of the coefficients in model 3 are significant, which did not occur in models 1 and 2. Since rachis cross-section only takes another minute per plant to measure, this equation is recommended if this slightly

improved degree of estimation is important in the study. It was used to estimate leaf area in section 4.6.3.

Although there was a good correlation between leaf biomass and both rectangular leaf area and rachis cross-sectional area, none of the prediction equations were completely satisfactory, especially when compared to the leaf area equations (Table 4.3). Clement & Habte (1994) reported a similar result with nursery grown seedlings. In their case, rachis cross-section was even less satisfactory, probably because they used mm, rather than tenths of mm. For the smaller bifid eophylls, one mm is a very coarse unit of measure.

Table 4.3. Allometric relationships between pejibaye bifid eophyll leaf area (L_A) and biomass (L_W) and various leaf dimensions. For each model a t-test is presented for each coefficient, and the standard error of the estimate (s), the coefficient of variation (CV = $s/\bar{Y}$), and the coefficient of determination (r^2) are presented. All models are significant at $p < 0.001$.

model	t_a	t_{b1}	t_{b2}	s	CV	r^2
1. $L_A = -13.3 + 1.14\ L_{RA}$	-1.2ns	48.6***		34.26	8.3	98.9
2. $L_A = 1.13\ L_{RA}^{0.995}$	1.2ns	54.9***		36.74	8.9	99.1
3. $L_A = -36.2 + 1.0L_{RA} + 5.0R_{W\cdot T}$	-2.5*	14.9***	2.2*	31.97	7.7	99.0
4. $L_W = -0.591 + 0.0139\ L_{RA}$	-1.5ns	16.3***		1.239	26.8	90.8
5. $L_W = -1.45+0.0087L_{RA}+0.19R_{W\cdot T}$	-2.8*	3.6***	2.3*	1.149	24.8	92.1
6. $L_W = 0.0095\ L_{RA}^{1.037}$	-18***	23.4***		1.309	28.3	95.3
7. $L_W = 0.0129\ L_{RA}^{0.83} \cdot R_{W\cdot T}^{0.333}$	-14***	6.6***	1.8ns	1.124	24.3	95.6

L_{RA} - rectangular leaf area; $R_{W\cdot T}$ - rachis width x thickness

4.5.2. The Pinnate Leaf

The study of leaf area was done separately from that of leaf biomass, because the original intent was simply to validate the leaf area allometrics, rather than expand the

study to new allometrics. When the validation procedure turned up new predictors of leaf area, more leaves were harvested and measured to provide an adequate sample size. The leaves for the leaf biomass study were taken from the first 10 trees sampled for the whole plant biomass study (leaves 2 and 3 from each tree). Consequently, the samples used are somewhat different and the results are presented separately.

4.5.2.1. Leaf Area

The dimensions of a random sample of pinnate leaves covering the range of rachis lengths typical of the pinnate-leaved sub-phase and the Pearson correlation coefficients calculated for each pair of variables are in Appendix B. Scatter plots of rachis length and rectangular leaf area versus measured leaf area (Figure 4.5), confirm the strong correlation between these pairs of variables and suggest their use for estimating leaf area.

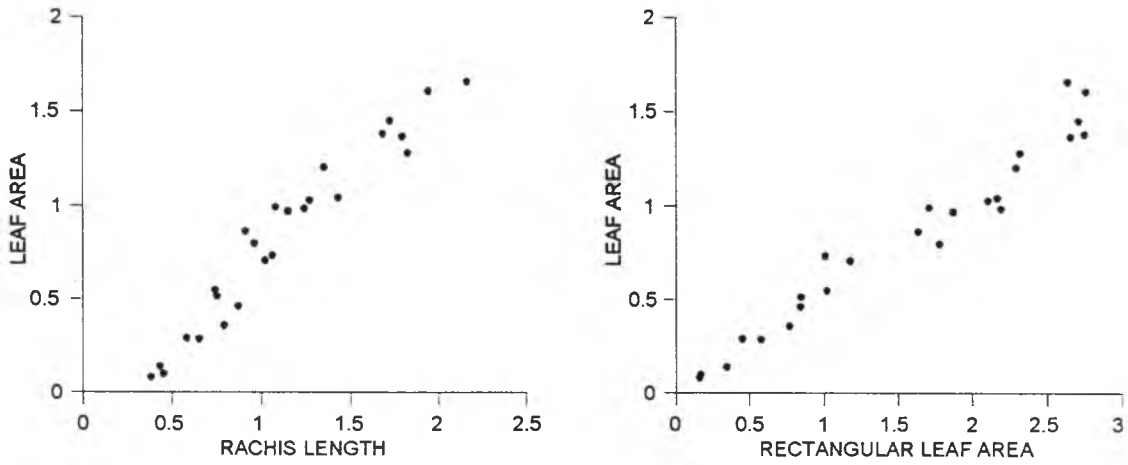

Figure 4.5. Scatter plots of rachis length (m) versus leaf area (m^2) and rectangular leaf area (= leaflet # x leaflet length (m) x leaflet width (m)) versus leaf area (m^2) for the 25 pejibaye leaves used to develop the leaf area allometric equations.

Each of the two most highly correlated variables was regressed individually against leaf area (Table 4.4). The rectangular leaf area (L_{RA}) estimated true leaf area (L_A) quite well (Table 4.4, model 1), as shown by the high r^2 and low s. The 'a' parameter, however, was not significantly different from zero. Model 2 decreased s and the CV somewhat. This form of the allometric, i.e., without the 'a' parameter, is that developed by Hardon et al. (1969) and Clement et al. (1985). The slope of the regression equation, 'b', is very similar to the 0.583 found by Clement et al. (1985) and 0.55 found by Hardon et al. (1990). To provide a more direct comparison, two of the goodness of fit parameters can be estimated for the equation of Clement et al. (1985) when used on the current data set: s = 0.1344; CV = 16.1. These parameters suggest that establishment phase pinnate leaves are slightly different from mature leaves, since both are larger than those of model 2.

Table 4.4. Allometric relationships between pejibaye pinnate leaf area (L_A) and various leaf dimensions. For each model a t-test is presented for each coefficient, and the standard error of the estimate (s), the coefficient of variation (CV = s/$\bar{Y}$) and the coefficient of determination (r^2) are presented. All models are significant at $p < 0.001$.

model	t_a	t_{b1}	t_{b2}	s	CV	r^2
1. $L_A = 0.0125 + 0.529\ L_{RA}$	0.30ns	22.8***		0.1004	12.0	95.6
2. $L_A = 0.535\ L_{RA}$		48.4***		0.0985	11.8	>95.6
3. $L_A = -0.207 + 0.923\ R_L$	-3.6***	19.6***		0.1163	13.9	94.1
4. $L_A = 0.299 + 0.354\ R_L^2$	5.2***	11.9***		0.1825	21.9	85.4
5. $L_A = -0.497 + 1.49R_L - 0.233R_L^2$	-4.3***	7.2***	-2.8**	0.1022	12.2	95.4
6. $L_A = 0.631*R_L^{1.67}$	-9.1***	15.7***		0.2241	26.8	91.1
7. $L_A = -0.113+0.304\ L_{RA}+0.421\ R_L$	-3.1***	6.6***	5.2***	0.0686	8.2	97.9

L_{RA} - rectangular leaf area; R_L - rachis length

The rachis length (R_L) also proved to be a reasonable estimator of L_A (Table 4.4, model 3) and was nearly as efficient as observed in African oil palm (Mendham 1971). Minitab's test of experimental error (Minitab 1994), however, detected a lack of fit and suggested that the data might display some curvature. The scatter plot of the model's residuals versus the model's predicted results showed a lack of residuals in both the upper left and upper right corners (graph not shown). To examine the possibility of curvature in greater detail, R_L^2 was tested as a variable (Table 4.4, model 4), with unsatisfactory results. When R_L and R_L^2 were used together, however, a more satisfactory model resulted (Table 4.4, model 5). To remove the effect of the hypothetical curvature, both variables were transformed by the natural logarithm, but the resulting power function (Table 4.4, model 6) proved to be less satisfactory.

The use of L_{RA} with R_L proved to be the best estimator of L_A (Table 4.4, model 7). Both variables provide significant information to the estimation, as shown by the significant t-tests. All of the goodness of fit parameters used here were optimized. No other combination of variables reduced s or increased r^2. This pair of variables also makes biological sense, perhaps the ultimate test of an allometric equation. The L_{RA} is a direct estimate of leaf area, although always a high estimate because it is based on the middle leaflet on the side with most leaflets. This is the reason that it works so well alone on mature plants (Hardon et al. 1969, Clement et al. 1985). The significance of R_L is more conjectural, but it can be considered as a modifier that accounts for the expansion of the leaf axis during the establishment phase of plant development.

4.5.2.2. Leaf Biomass

The dimensions of a random sample of pinnate leaves covering the range of rachis lengths typical of the pinnate sub-phase and Pearson correlation coefficients calculated for each pair of variables are in Appendix B. Scatter plots of rachis thickness and leaf area versus leaf biomass (Figure 4.6) confirm the strong correlation between these pairs of variables and suggest their use for estimating leaf biomass. Although the data points are somewhat clumped in Figure 4.6., they do cover most of the range of variation observed in the pinnate sub-phase.

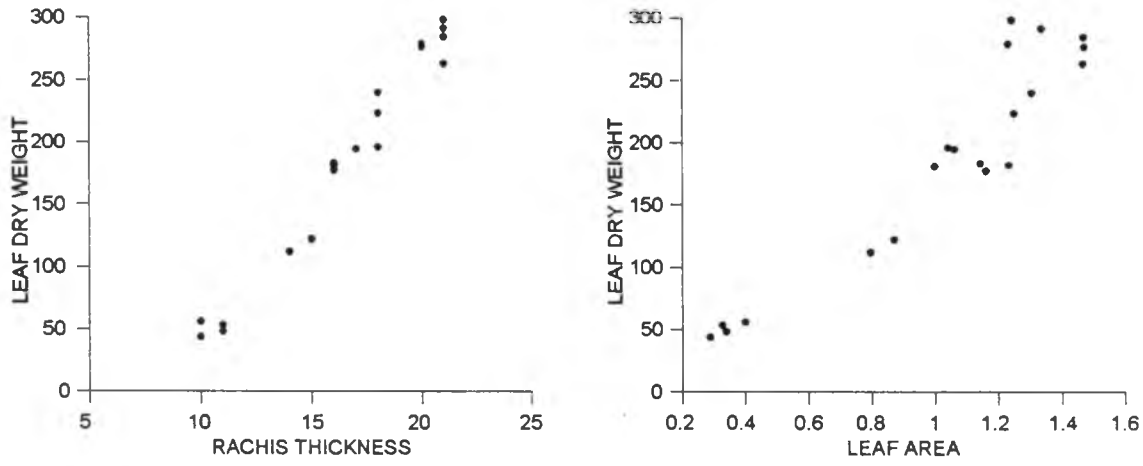

Figure 4.6. Scatter plots of rachis thickness (mm) versus leaf biomass (g) and leaf area (m^2) versus leaf biomass for the 20 pejibaye pinnate leaves used to validate the leaf biomass allometric equations.

All of the rachis dimensions were regressed against leaf biomass to determine their relations with this trait (Table 4.5), even though the correlation did not look very promising. The rachis thickness at the insertion of the first leaflet yields the best allometric relationship with leaf biomass (Table 4.5, model 4). This is different from Clement et al.'s (1990) mature leaf biomass estimator, which used a combination of

rachis cross-sectional area and estimated leaf area similar to model 7, which is the second best estimator. Corley et al. (1971) found that the cross-sectional area alone was sufficient in African oil palm, similar to model 6. Squares and natural logarithms failed to improve the goodness of fit parameters.

Table 4.5. Allometric relationships between pejibaye leaf biomass (L_W) and various leaf dimensions. For each model a t-test is presented for each coefficient, and the standard error of the estimate (s), the coefficient of variation (CV = $s/\overline{Y}$), and the coefficient of determination (r^2) are presented. All models are significant at $p < 0.001$.

model	t_a	t_{b1}	t_{b2}	s	CV	r^2
1. $L_W = -56.6 + 188\ R_L$	-1.4ns	6.1***		51.23	27.6	65.5
2. $L_W = 148\ R_L$		16.9***		52.39	28.2	<65.5?
3. $L_W = -124 + 16.1 R_W$	-7.6***	19.5***		18.99	10.2	95.3
4. $L_W = -193 + 22.9\ R_T$	-12***	25.2***		14.87	8.0	97.1
5. $L_W = -5.55 + 57.0\ R_{W \cdot T}$	-0.6ns	21.7***		17.18	9.3	96.1
6. $L_W = 55.6\ R_{W \cdot T}$		53.9***		16.88	9.1	>96.1
7. $L_W = -15.8 + 44.6 R_{W \cdot T} + 51.0 L_A$	-1.5ns	6.7***	2.0ns	15.91	8.6	96.7

R_L - rachis length; R_W - rachis width; R_T - rachis thickness; $R_{W \cdot T}$ - rachis width x thickness; L_A - leaf area.

The same data set can be used to identify an allometric relationship between leaf dimensions and leaflet biomass, since the latter trait is useful for calculating specific leaf area (Table 4.6). In this case, the cross-sectional area of the rachis at the insertion of the first leaflet gives an excellent relationship with leaflet biomass (Table 4.6, model 6). The best model, however, is model 7, which combines cross-sectional area with estimated leaf area. Forcing the intercept through zero did not improve any of the goodness of fit

parameters. Since leaf area will normally be estimated anyway for growth studies in pejibaye for heart of palm, model 7 is indicated.

The importance of the rachis dimensions at the point of insertion of the first leaflet make biological sense. Tomlinson (1990) has shown that the mechanics of the palm leaf are such that the petiole can be equated with a cantilever that holds the leaf blade in relation to the leaf sheath and plant stem. At some point along the petiole is the critical point where the cross-sectional dimensions are most closely related to the leaf blade weight and, theoretically, leaf area. In African oil palm, this point is right at the insertion of the first leaflet, since the r^2 of the relationship is 99% (Corley et al. 1971). In pejibaye, however, the critical point is apparently not precisely at the insertion of the first leaflet, since the r^2 of the relationship is lower than 97% at best (Tables 4.5 and 4.6).

Table 4.6. Allometric relationships between pejibaye leaflet biomass (LL_W) and various leaf dimensions. For each model a t-test is presented for each coefficient, and the standard error of the estimate (s), the coefficient of variation ($CV = s/\bar{Y}$), and the coefficient of determination (r^2) of the model are presented. All models are significant at $p < 0.001$.

model	t_a	t_{b1}	t_{b2}	s	CV	r^2
1. $LL_W = -27.5 + 90.3\ R_L$	-1.6ns	7.1***		21.16	23.9	72.0
2. $LL_W = 70.6\ R_L$		19.2***		22.03	24.8	<72.0?
3. $LL_W = -54.7 + 7.47\ R_W$	-8.9***	24.1***		7.134	8.0	96.8
4. $LL_W = -84.7 + 10.5\ R_T$	-11***	23.7***		7.227	8.1	96.7
5. $LL_W = 0.63 + 26.3\ R_{W\cdot T}$	0.2ns	24.2***		7.093	8.0	96.9
6. $LL_W = 26.4\ R_{W\cdot T}$		62.5***		6.908	7.8	>96.9?
7. $LL_W = -6.37+17.8\ R_{W\cdot T}+34.7\ L_A$	-1.9ns	8.3***	4.3***	5.074	5.7	98.4

R_L - rachis length; R_W - rachis width; R_T - rachis thickness; $R_{W\cdot T}$ - rachis width x thickness; L_A - leaf area.

4.5.3. The Whole Plant

Descriptive statistics of the dimensions of a random sample of 27 plants covering the range of plant heights typical of the establishment and early adult vegetative phases and Pearson correlation coefficients calculated for each pair of variables are in Appendix B. Scatter plots of plant height versus whole plant leaf area and biomass (Figure 4.7) confirm the strong correlation between these variables and suggest their use for estimating whole plant dimensions. In both figures, however, there appear to be two populations. The population that has plant heights of 0-0.25 m, representing the bifid eophyll sub-phase, appears to have a different slope from the population that has plant heights of 0.25-1.7 m. Consequently, three sets of regressions were done: A. using the entire data set (n = 27); B. using only the bifid eophyll sub-phase plants (n = 9); C. using only the pinnate leaved sub-phase plants (n = 18).

The degree of variability evident for leaf area and biomass (Figure 4.7) is somewhat different. Whole plant leaf area is apparently more variable than whole plant biomass, which suggests that the prediction equations will be more variable for the former than the latter. This is commonly observed in dicots also. Harrington & Fownes (1993), for example, obtained lower r^2's for their whole plant leaf area allometrics than for their whole plant biomass allometrics in four tropical tree species.

The whole plant leaf area allometric equations developed for all plants (Table 4.15.A) presented various anomalies or inadequacies. The equations that used height, with or without leaf number or its square, presented the best precision, with CV's in the low 20s. These equations, however, occasionally gave negative leaf area estimates, especially for

the small plants. Consequently, none of them are useful for predicting leaf areas in the field. The power functions did not have this failure, but had much higher CV's. A possible conclusion is that there are two allometric relationships in this data set.

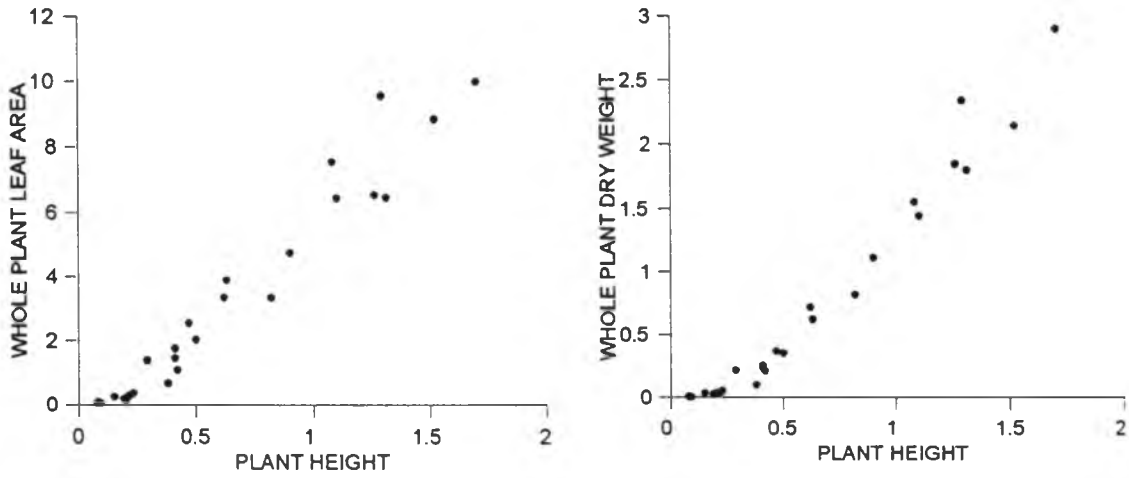

Figure 4.7. Scatter plots of plant height (m) versus whole plant leaf area (m^2) and biomass (kg) for the 27 pejibaye plants used to study whole plant allometric relations. If 0.25 m is taken as the cut-off between the bifid eophyll and pinnate establishment sub-phases, it appears that there are two discrete populations, with different slopes.

The t-test to compare models B.1 and C.1 was insignificant, but was close to significance, suggesting a tendency in this direction. Since there are also biological reasons for separating the two sub-phases, i.e., they have different leaf forms - bifid and pinnate, each section of the data set was analyzed separately.

The bifid eophyll sub-phase allometric equations that included height, alone or with either leaf number or its square, did not produce negative leaf area estimates this time (Table 4.7, models B.1-3). The power function using both variables (Table 4.7, model B.5), however, not only avoided negative estimates but had a lower s, suggesting that this

equation is more precise. The small number of plants used to generate this equation suggests that it must be used with caution until further work is done to validate it.

Table 4.7. Allometric relationships between pejibaye whole plant leaf area (L_A) and height (H) or leaf number (L), their squares, and their natural logarithms, in various combinations. A. All plants (n = 27). B. Only bifid eophyll plants (n = 9). C. Only pinnate-leaved plants (n = 18). For each model a t-test is presented for each coefficient, and the standard error of the estimate (s), the coefficient of variation (CV = $s/\bar{Y}$), and the coefficient of determination (r^2) are presented. All models are significant at $p < 0.001$.

model	t_a	t_{b1}	t_{b2}	s	CV	r^2
A.1. $L_A = -0.852 + 6.49\ H$	-3.8***	22.7***		0.7139	23.1	95.2
A.2. $L_A = -1.67 + 6.29\ H + 0.148\ L$	-2.2*	19.0***	1.1ns	0.7092	22.9	95.3
A.3. $L_A = -1.22 + 6.28\ H + 0.0120\ L^2$	-3.3**	19.2***	1.3ns	0.7052	22.8	95.3
A.4. $L_A = 5.64 \cdot H^{1.76}$	18.1***	23.9***		1.3468	43.6	95.7
A.5. $L_A = 0.93 \cdot H^{1.66} \cdot L^{0.94}$	-0.1ns	21.4***	2.5*	1.7909	58.0	96.4
B.1. $L_A = -0.0687 + 1.69\ H$	-1.5ns	5.6***		0.05373	29.8	79.4
B.2. $L_A = -0.284 + 1.74\ H + 0.0382\ L$	-2.7*	7.2***	2.2ns	0.04321	24.0	86.7
B.3. $L_A = -0.186 + 1.77\ H + 0.0035\ L^2$	-2.9*	7.3***	2.2ns	0.04295	23.8	86.9
B.4. $L_A = 3.35 \cdot H^{1.58}$	2.1ns	5.7***		0.0539	29.9	80.0
B.5. $L_A = 0.157 \cdot H^{1.61} \cdot L^{1.85}$	-2.7*	12.0***	4.8**	0.0328	18.2	95.2
C.1. $L_A = -1.07 + 6.69\ H$	-2.4*	14.3***		0.8568	18.8	92.3
C.2. $L_A = -2.28 + 6.52\ H + 0.200\ L$	-1.9ns	13.3***	1.1ns	0.8500	18.7	92.4
C.3. $L_A = 5.37 \cdot H^{1.38}$	22.0***	11.3***		0.8352	18.4	88.1
C.4. $L_A = 2.94 \cdot H^{1.36} \cdot L^{0.313}$	1.4ns	10.7***	0.8ns	0.8593	18.9	87.8

The pinnate sub-phase allometric equations with non-transformed variables also avoided negative leaf area estimates (Table 4.7, models C.1-2). By forcing leaf number into the equation with height, s was reduced by a small fraction. The power function with height alone (Table 4.7, model C.3) had a lower s that suggests a better fit. The power function with height and leaf number (Table 4.7, model C.4) had a slightly higher s than model C.3, probably because neither a nor b_2 are significant. There is a biological reason

for including leaf number in the equation: the plants used to generate these equations had a lower than expected leaf number and its inclusion may help expand the utility of the model to normally leaved plants.

Although the height versus whole plant biomass scatter plot (Figure 4.7) looks less variable than the height versus leaf area plot, the biomass allometric equations generated for all plants (Table 4.8) were no less variable than were the leaf area equations. The very high CV's produced by the power functions again suggest that there are two populations in the data set. In fact, the slopes of models B.1 and C.1 were significantly different ($p < 0.05$) when compared by a t-test, suggesting that the trend observed for leaf area also exists in reality. This result provides further justification for dividing the establishment phase into two distinct sub-phases based on leaf form.

In the case of the bifid eophyll sub-phase plants, the non-transformed variables proved to be no more efficient at predicting biomass (Table 4.8, models B.1-3) than was the case for all plants taken together. The power function with both height and leaf number improved precision significantly (Table 4.8, model B.5). Additionally, all parameters in the equation were important.

For pinnate-leaved sub-phase plants the use of height alone provided the best prediction of whole plant biomass (Table 4.8, model C.1). When leaf number was forced into the equation, s improved slightly, even though leaf number was not an important contributor to the prediction equation. The power function did not improve the precision of prediction, but gave better predictions at the small end of the data set where errors are more critical if the equations will be used for growth analysis, as in Chapter 5. Szott et al.

(1993) found a prediction equation with nearly the same coefficient of determination: $W = -0.24 + 0.024L^2 + 0.02D^2$, where D was measured at 65 cm above soil level. For plants in the pinnate-leaved sub-phase, it is unclear what the diameter of the crown shaft at 65 cm means, especially for smaller plants. Once the stem has appeared and elongated to this height, i.e., the adult vegetative phase, the meaning is clear. Height, however, has a clear meaning at all times, providing a sounder biological basis for the equation during this sub-phase.

Table 4.8. Allometric relationships between pejibaye whole plant biomass (W) and plant height (H) or leaf number (L), their squares, and their natural logarithms, in various combinations. A. All plants (n = 27). B. Only bifid eophyll plants (n = 9). C. Only pinnate-leaved plants (n = 18). For each model a t-test is presented for each coefficient, and the standard error of the estimate (s), the coefficient of variation (CV = s/$\bar{Y}$), and the coefficient of determination (r^2) are presented. All models are significant at $p < 0.001$.

model	t_a	t_{b1}	t_{b2}	s	CV	r^2
A.1. $W = -0.330 + 1.72\,H$	-6.1***	24.4***		0.1755	24.7	95.8
A.2. $W = 0.0797 + 1.05\,H^2$	1.9ns	25.3***		0.1700	23.9	96.1
A.3. $W = -0.131 + 0.839H + 0.556H^2$	-2.1*	4.0***	4.3***	0.1348	19.0	97.5
A.4. $W = 1.266 \cdot H^{2.07}$	2.7*	30.3***		0.2796	39.4	97.2
A.5. $W = 0.181 \cdot H^{1.96} \cdot L^{1.01}$	-2.6*	28.5***	3.0**	0.4306	60.6	97.9
B.1. $W = -0.0142 + 0.264\,H$	-2.2ns	6.5***		0.00735	36.5	83.5
B.2. $W = 0.95\,H^2$		12.6***		0.00685	34.0	>84.2
B.3. $W = -0.031 + 0.27H + 0.00049L^2$	-3.6*	8.6***	2.4ns	0.00570	28.5	90.1
B.4. $W = 0.797 \cdot H^{1.90}$	-0.4ns	6.5***		0.0071	35.5	83.7
B.5. $W = 0.027 \cdot H^{1.93} \cdot L^{2.05}$	-5.9***	16.3***	6.1***	0.0038	19.0	97.3
C.1. $W = -0.557 + 1.92\,H$	-6.7***	22.0***		0.1600	15.1	96.6
C.2. $W = -0.850 + 1.88H + 0.0484L$	-4.0***	21.1***	1.5ns	0.1542	14.5	96.8
C.3. $W = 1.22 \cdot H^{1.77}$	2.8*	15.5***		0.1718	16.2	93.4

4.6. Conclusions

The morphological description of the seedling, establishment and early adult vegetative phase plants identified three major factors for future consideration: 1) changes in the expression of spines on the petiole during plant development; 2) a lower than expected leaf number at first harvest; and 3) a strong reduction in offshoot number from the first to the second harvest.

While the developmental change in spine expression will be unimportant in Hawai'i, where spines will be eliminated as a first priority of an improvement program, adequate characterization of pejibaye germplasm requires consideration of this developmental change. Current pejibaye descriptors (Clement 1986a) are inadequate because they characterize spines on mature plants, after the developmental change has acted. Consequently, spines should be characterized on immature plants, at six months after field planting.

Current notions of palm growth (Tomlinson 1990) predicted that harvest size pejibayes should have up to 17 leaves. At first harvest, leaf number averaged 6.6, less than half the expected number. It is important to determine the reasons for this departure from expectation. Micro-nutrient deficiency was raised as a possible reason for reduced leaf number. While no data are currently available, circumstantial evidence (more leaves when younger plants received a micro-nutrient supplement, fewer leaves when older plants did not) suggests that micro-nutrient deficiency should receive more research attention. A higher leaf number should result in faster growth, with obvious benefits for the producer.

The average number of offshoots at first harvest was three times the number at second harvest. The reasons for this extreme reduction require more study. Offshoot management probably played an important role in this reduction in this project, allowing much greater within-clump competition than is practiced commercially. Further observations are important to determine the long-term trends in offshoot production, as the offshoots are critical to the long-term survival of the plantation.

Previously developed prediction equations for bifid eophyll leaf area and pinnate leaf area and biomass were generally validated, but slight improvements were found for each. A prediction equation for bifid eophyll biomass was developed. The recommended prediction equations are:

Bifid eophyll:

$$L_A = -36.2 + 1.0L_{RA} + 5.0R_{W \cdot T}$$

$$L_W = 0.0129\, L_{RA}^{0.83} \cdot R_{W \cdot T}^{0.333}$$

Pinnate leaf:

$$L_A = -0.113 + 0.304\, L_{RA} + 0.421\, R_L$$

$$L_W = -193 + 22.9\, R_T$$

The proposal of two sub-phases within the establishment phase defined by Tomlinson (1990) was shown to be both biologically and statistically valid. Biologically, leaf form changes from bifid eophyll to pinnate as the plant passes through the height interval of 0.2 to 0.3 m, rarely to 0.4 m. Statistically, the slopes of the regressions that relate height to whole plant biomass were significantly different for the two sub-phases. Although the

slopes of the regressions that relate height to leaf area suggest the same difference, they were not statistically different.

Plant height is defined as the best predictor of both whole plant leaf area and biomass in both sub-phases. Leaf number is often a useful auxiliary predictor. The prediction equations that are used to study growth in Chapter 5 are:

Bifid eophyll sub-phase:

$$L_A = 0.157 \cdot H^{1.61} \cdot L^{1.85}$$

$$W = 0.027 \cdot H^{1.93} \cdot L^{2.05}$$

Pinnate-leaved sub-phase:

$$L_A = 2.94 \cdot H^{1.36} \cdot L^{0.313}$$

$$W = 1.22 \cdot H^{1.77}$$

All four whole plant prediction equations must be used with caution. Not only is the sample size used to generate them small, especially for the bifid eophyll sub-phase predictors, but the plants used to generate the equations had fewer leaves than expected. Consequently, these equations must be validated before use in other projects.

Chapter 5. Growth and Yield of Pejibaye at High Density

5.1. Introduction

Variation in growth rates among individuals in natural populations is the norm and has been observed in both natural populations of palms (Tomlinson 1990) and in the economically important cultivated palms (Child 1974, Hartley 1977). While much of this variation may be environmental (Coleman et al. 1994), there is always significant genetic variation that can be exploited in improvement programs (Gupta 1992). The objectives of this chapter are to describe the variation in growth and yield of pejibaye progenies at high density, both from seed and from suckers, and to determine the effects of plant density on yield and selected growth parameters.

Coleman et al. (1994) emphasize the importance of comparing phenotypic variation across both uniform time intervals and uniform developmental stages. Phenotypic traits change throughout growth and development of individuals and rates of change are variable also. Most growth studies have been done with the important annual crops. With short growing seasons and highly selected crop varieties, age and developmental stage are highly correlated, so that little attention is focused on the comparison recommended by Coleman et al. (1994). With perennials, however, this comparison can provide greater insight into individual and population variation across environments. This variation, in turn, can be exploited or modified by agronomists and geneticists to maximize economic yield.

In African oil palm (*Elaeis guineensis* Jacq.), Corley et al. (1971) found that there are individuals and progenies that are good competitors and others that are poor competitors.

The highly competitive plants produce more leaf area and require lower planting densities to attain maximum fruit yield. The poorly competitive plants produce less leaf area to yield well and can be planted at higher density without reducing fruit yield. The poor competitors tend to have a higher harvest index than the good competitors and to produce more on a per hectare basis.

In terms of adaptive strategies, the good competitors partition more photoassimilates to occupying space (large leaves, both in length and area, and rapid stem elongation) and yield well as individuals. In contrast, the poor competitors partition less photoassimilates to occupying space and more to the reproductive effort, so they yield well in high density populations. For the African oil palm breeder, the second strategy is preferable. For the pejibaye breeder, however, the competitive strategy may be preferable for production of palm heart, since purely vegetative growth is the objective. The main question to answer is if the competitive strategy functions well at high density for purely vegetative growth. Unfortunately, little is currently known about growth of pejibaye at high density.

In coconut (*Cocos nucifera* L.), Liyanage (1967) observed that rapid germination and establishment (as measured by leaf production) is correlated with precocity (time from planting to fruiting) and the latter is correlated with mature yield. Both leaf production and precocity had moderate heritabilities, and considerable genetic variation was present. In African oil palm, Blaak (1972) found moderate heritabilities and considerable variation for precocity, measured as months from field planting to the time at which 30% of plants in each progeny were fruiting. Although Blaak did not examine growth in detail,

precocity in palms implies rapid growth, since flower initiation only occurs after the plant has attained the adult reproductive phase (Tomlinson 1990).

Corley (1983) recommended the use of growth analysis as a tool to improve the efficiency of perennial crop improvement programs. Growth analysis can help identify specific traits or parameters that contribute to economic yield, as well as quantify the phenotypic variation present in the population. Corley (1973), for example, found that African oil palm vegetative dry matter production is less affected than fruit yield as density increases. Tomlinson (1990) suggests that palms in general follow the same trend. If pejibaye does also, high density might not reduce yield of heart of palm, since the latter is simply a small fraction of the vegetative dry matter production. Clement et al. (1988) reviewed agronomic research on pejibaye for heart of palm to identify plant responses to high density (2,500 to 10,000 plants/ha). Two important results were decreases in both the percentage of plants harvested (% cut) and the number of offshoots produced at high density, suggesting that long-term individual plant yield and replacement are affected by density.

Yield results from Costa Rica are difficult to compare with Brazilian results because of the way yield data were presented. The Costa Ricans used field-ready hearts, rather than export-quality hearts, as their measure of yield. Yields in excess of 3 t/ha were reported (Zamora F 1985), but only a fraction (20-30%) of this was of export quality. The Brazilians, on the other hand, used export-quality yield and reported 1.2 t/ha at 5000 plants/ha (Moreira Gomes & Arkcoll 1988) and 1.6 t/ha at 10,000 plants/ha (Moreira

Gomes et al. 1988). The latter authors reported a decrease in average heart weight as density increased.

The current study uses classical growth analysis procedures to describe the growth and variation in growth habit of seven pejibaye progenies at Ninole. There were problems with field establishment at Poamoho and Waiakea and results are not reported from these sites. Germination is included in the description of growth because there may be interesting correlations with later growth. Growth was examined as a function of time and development phase. Yields are presented on a per plant and per hectare basis. The effects of density on growth and yield are examined for the first harvest cycle.

5.2. Germination

The viability of the seed, estimated by flotation, was slightly lower than expected (Table 5.1). Less than 5% of fresh pejibaye seed should float, indicating inviability (S.A.N. Ferreira, personal communication). Germination averaged only 53%, versus an expected 80-85% for fresh, good quality seed (Ferreira & Santos 1992). Only one progeny, B-2, attained the expected level of germination. The emergence velocity index (EVI) ranged from 0.1 for the progeny with the lowest germination percentage to 2.17 for the highest. A high EVI occurs when seed of a progeny germinates rapidly (Figure 5.1). Progenies B-6 and B-8, for example, had nearly identical germination percentages, but B-6 had a higher EVI because it germinated rapidly. A high EVI also suggests a lack of dormancy, while a low EVI suggests the presence of dormancy (Copeland & McDonald 1985). Seedlings were transplanted to the nursery 30 days after the end of germination

observations. At transplant, the number of bifid eophylls ranged from 0 (just the second sheath) to three. This variation can be viewed as growth based on seed reserves, since the seedlings still had relatively rudimentary root systems and the germination substrate lacked nutrients for plant growth.

Table 5.1. Seed viability, mean seed weight, germination percentage, emergence velocity index (EVI), and seedling dimensions at transplant to nursery of the 10 Benjamin Constant pejibaye progeny seed lots received from Brazil.

U.H. id #	Viability[a] (%)	Seed Wt. (g)	Germ. (%)	EVI	No. of Leaves	Length[b] (cm)
B-0	85.8	2.3	51.0	1.23	1.87	16.3
B-1	86.2	2.1	77.5	1.83	1.74	14.2
B-2	85.8	1.7	80.5	2.17	2.01	14.8
B-3	82.6	1.9	64.0	1.48	1.78	14.8
B-4	92.6	2.5	5.0	0.10	1.78	14.6
B-5	83.3	1.9	52.5	1.51	1.95	15.5
B-6	87.2	2.1	68.5	1.78	1.93	15.0
B-7	66.6	2.9	15.5	0.28	1.26	14.0
B-8	96.2	2.8	70.0	1.50	1.83	16.5
B-9	89.6	1.9	51.0	1.20	1.70	16.8
mean	85.6	2.2	53.6	1.31	1.78	15.2

[a] Apparent viability obtained by floating seeds in 20 liters of water (total - floaters)
[b] Leaf length

Viability was not correlated with days from harvest to sowing (Table 5.2); a negative correlation, however, would have been expected, as the longer the interval between seed harvest and sowing, the lower the viability (Ferreira & Santos 1992). Germination percentage was positively correlated with days to sowing ($r = 0.80^{**}$), the opposite of what was expected. This appears to be due to the progenies, B-4 and B-7, with the lowest

germination. These two progenies may have been dried too fast, as Ferreira & Santos (1993) have shown the detrimental effects of rapid drying on seed quality. There was a significant correlation (r = 0.67*) between EVI and the number of bifid leaves at transplant, since early germination allows more time for differentiation and expansion of leaves.

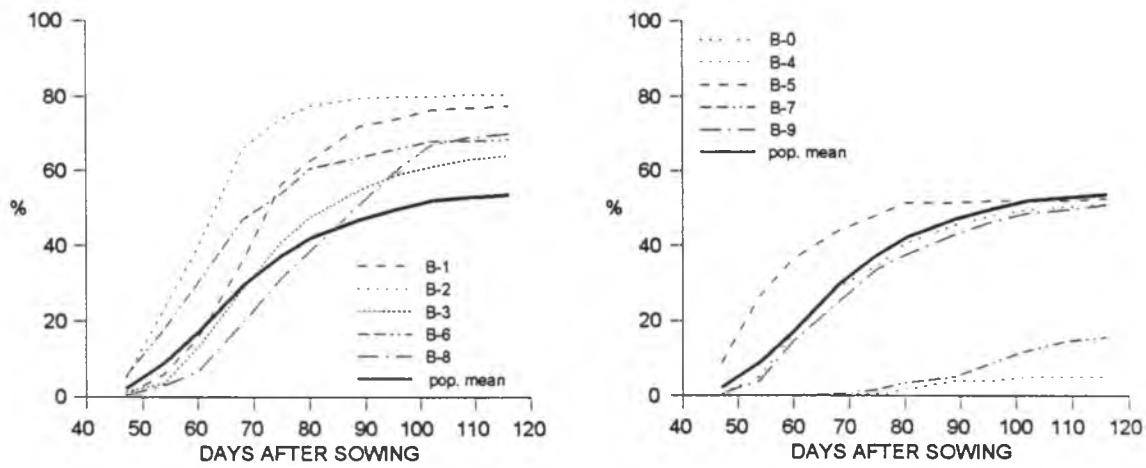

Figure 5.1. Cumulative germination of pejibaye progenies over time. Left. The five progenies with the highest final germination percentage. Right. The five progenies with the lowest final germination percentage.

5.3. The Effect of Density

The densities used in this study bracket the 5000 plants/ha density used in commercial plantations of pejibaye for heart of palm in Costa Rica. All of the analyses of growth carried out in this chapter were based on a split-plot design, with densities as the main plots. Since density was expected to be a significant environmental effect, it was examined first.

Table 5.2. Correlation matrix of progeny seed quality, germination results and plant size at transplant to the nursery.

	Storage[a]	Viability[b]	Seed Wt.	Germ. %	EVI[c]	Leaf #
Viability	0.24					
Seed Wt.	-0.56	-0.17				
Germ. %	0.80	0.31	-0.55			
EVI	0.81	0.28	-0.66	0.98		
Leaf #	0.57	0.68	-0.63	0.59	0.67	
Leaf (cm)	0.21	0.55	-0.07	0.21	0.17	0.34

Critical values of r are: 0.6319, 0.7646, 0.8721, for p = 0.05, 0.01, 0.001, respectively.
[a] Seed storage time in days from fruit harvest to sowing.
[b] Apparent viability obtained by floating seed in 20 liters of water (total - floaters)
[c] Emergence velocity index

The effect of plant position within the plot was tested by treating the up-hill outside row, the center row, and the down-hill outside row as sub-sub-plots, and analyzing the trial as a split-split-plot (Little & Hills 1978). No significant differences were detected for position effects on plant height and leaf number (Appendix C), nor offshoot number, whole plant leaf area and biomass, nor for relative growth rate (RGR), unit leaf rate (E_A), and leaf area ratio (LAR) (data not shown), at any density. This lack of significant differences was unexpected.

There are four possible reasons for the lack of position effects on individual plant dimensions: 1) the 9-plant plots were too small, allowing sufficient light to penetrate the plot from the row spaces; 2) palm growth was sufficiently irregular that most plants received sufficient light from some side; 3) the reduced leaf number noted in Chapter 4 reduced leaf area index (LAI) and therefore competition; or 4) the densities studied do not significantly affect vegetative growth of pejibaye. The first reason was recognized as

a possible limitation before field planting. The absolute lack of sufficient germplasm, because of poor germination, did not permit larger plots. The second reason is probably important also, as each plant has a unique growth rate that is affected by its unique micro-environment. Also, as harvest progressed, the canopy became even more irregular as some plants were removed each month. The third reason certainly existed during the experimental period, although the magnitude of its effect on LAI is unknown. The older leaves, which may have died earlier than with adequate micronutrients, would have a small impact on LAI because they were small. The fourth reason is theoretically possible since Corley (1973, 1976b) observed that vegetative growth in African oil palm was not reduced at higher than optimum fruiting densities, while fruit yield dropped dramatically at above optimum densities.

The lack of position effect allowed the use of all of the plants in each plot, rather than only the single center plant or center row. Consequently, data from all plants were used for all subsequent analyses, enhancing the statistical power of the mean comparisons and, in Chapter 6, permitting a better estimate of phenotypic and genotypic variances.

Mean crop growth rate (CGR) and LAI presented significant density differences, but these were expected because they are density dependent parameters. CGR and LAI increased linearly over the densities studied (Figure 5.2). There was no indication that values for these two vegetative growth parameters were approaching a limit. Some progenies behaved differently at higher density than at lower, although the progeny x density interaction was not significant.

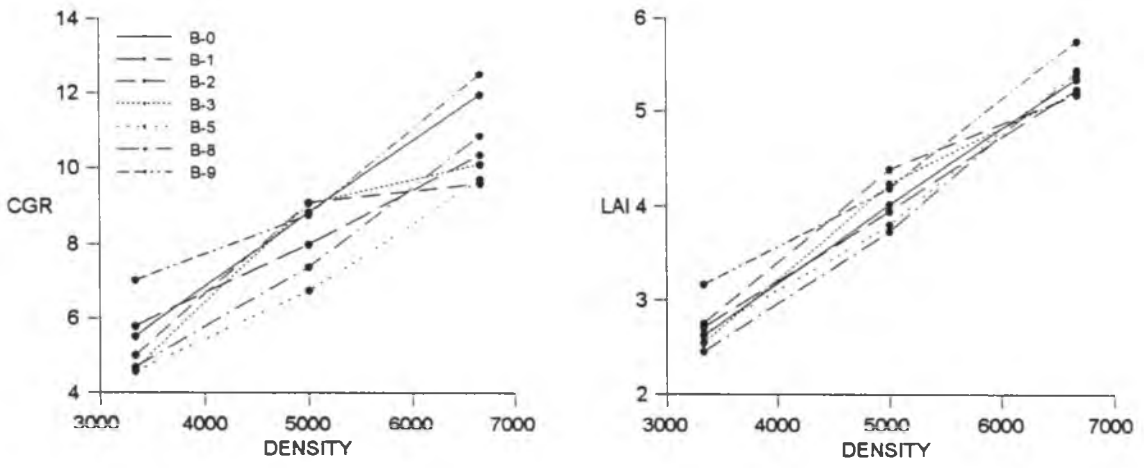

Figure 5.2. The effect of planting density on mean crop growth rate (CGR from field planting to first harvest (t/ha/yr) - Left) and mean leaf area index (LAI - Right) of pejibaye up to first harvest at Ninole.

Progenies B-2 and B-3 had similar CGR's at 5000 and 6666 plants/ha, suggesting that they might be affected by the higher density. Progeny B-9 had a higher CGR than the others at both low and high density, but not at 5000 plants/ha. Similar variation was observed with respect to LAI, although it was less pronounced.

Both potential and actual yields presented significant progeny differences. The differences between potential (assuming all plants harvested during the year) and actual yields are due principally to pig damage in March 1994, although a small percentage of plants in each progeny also did not attain harvest size by 1050 days in the field. Most progenies presented yield increases that were nearly linear across the densities used (Figure 5.3). Progeny 9 presented a strong elbow, due to a decrease in heart of palm weight at 5000 plants/ha, for reasons that are unclear.

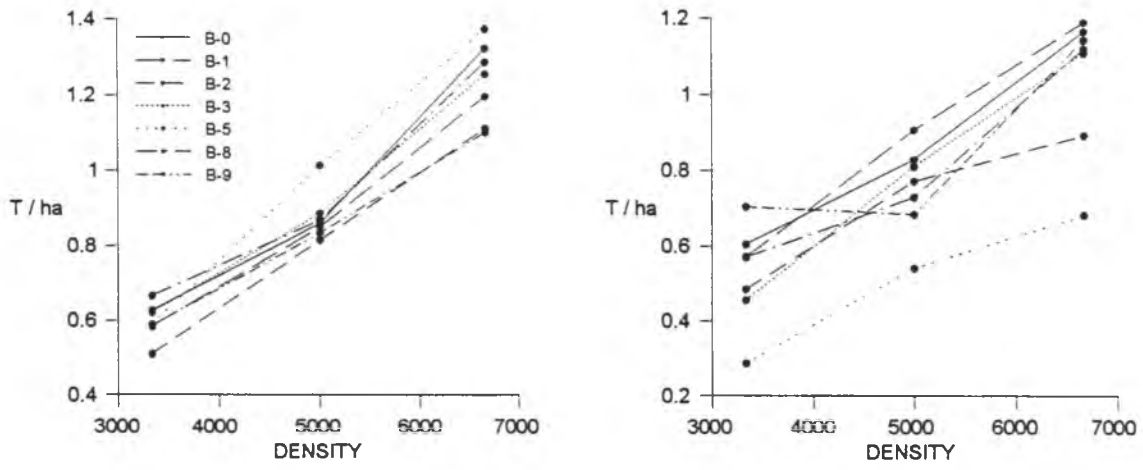

Figure 5.3. Effect of density on pejibaye potential heart-of-palm yields (Left) and actual heart-of-palm yields (Right) at conclusion of the first harvest at Ninole.

After 18 months of harvesting, most plants had been harvested at least once (including those damaged by pigs). The 18-month total yield, based on number of stems harvested per clump, weight of each heart, and corrected for the number of clumps harvested, gives a better idea of the long-term potential of the progenies (Figure 5.4, Left). Only progeny B-5 was significantly different from the others. There was no density effect on single plant growth rate when viewed as the number of stems harvested from a clump during the 18 months of harvest (Figure 5.4, Right).

Days to harvest (DTH) was not significantly effected by density, nor were progeny by density interactions significant. Progenies were significantly different at each density and some grew somewhat differently at low than at high density (Figure 5.5). Progeny B-1 was the only progeny that followed the expected trend, more days to harvest at higher density, that was observed as a reduction in % cut in Clement et al.'s (1988) reanalysis of Zamora's (1985) study. Three progenies, B-0, B-5, and B-8, attained harvest size more

rapidly at high density than at low. Two progenies, B-2 and B-3, attained harvest size more rapidly at 5000 plants/ha than at either 3333 or 6666 plants/ha. Progeny B-9 was the opposite, attaining harvest size more rapidly at high and low density and more slowly at intermediate density.

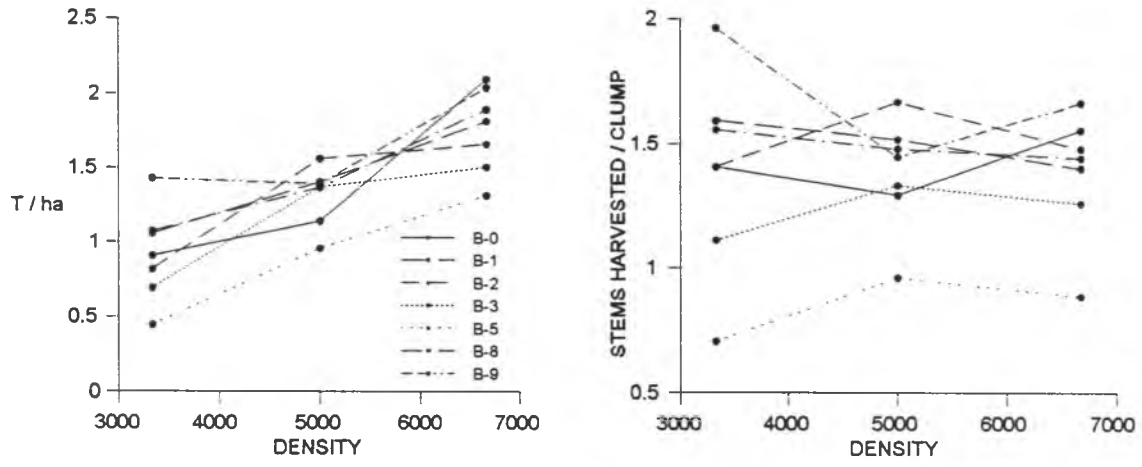

Figure 5.4. Effect of density on pejibaye total actual heart of palm (Left) and mean number of stems harvested per clump (Right) after 1050 days (18 monthly harvests) at Ninole.

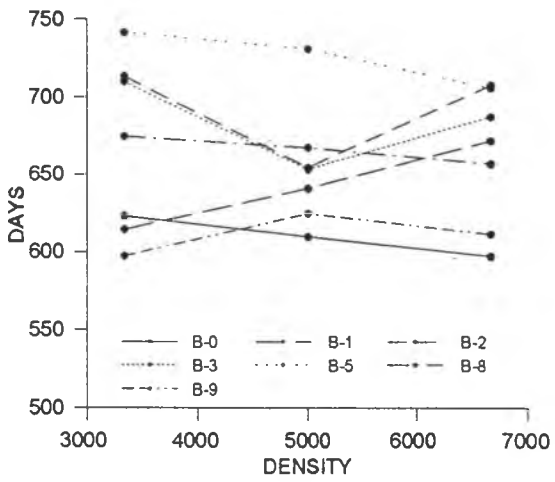

Figure 5.5. The effect of density on mean number of days from first evaluation to first harvest for pejibaye progenies at Ninole.

Although there were no significant differences among densities for heart of palm weight and length and no significant progeny x density interactions, progenies were significantly different at each density and some progenies were different at different densities (Figure 5.6). Many of the progenies had smaller hearts at the 5000 plant/ha density. Why an intermediate density would present a decrease in weight or length is unclear, but was also observed in Clement et al.'s (1988) reanalysis of Zamora's (1985) density study in Costa Rica. Since the density effects and progeny x density interactions were statistically insignificant, it is unclear how much importance should be given to this variation when attempting to identify individuals and progenies as parental material for future breeding work.

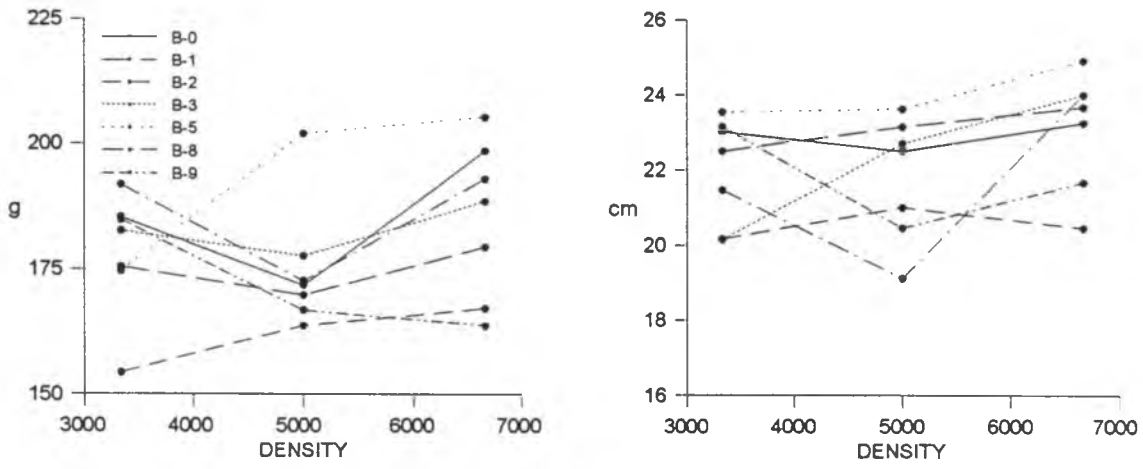

Figure 5.6. Effect of density on pejibaye heart-of-palm weight (Left) and length (Right) during the first harvest cycle at Ninole.

5.4. Yield

Three products can be obtained from a single stem of pejibaye: the edible stem, which extends from the apical meristem down to 10-25 cm below the meristem; the heart of

palm, composed of the tender immature leaves wrapped within a tender petiole sheath; and the edible leaf, composed of the tender immature leaves above the enveloping petiole sheath. In Latin America, the heart of palm is the only product with a large market at this time. Occasionally the edible stem is diced and marinated, usually mixed with short chunks of heart. The market for this product is expanding. Export heart-of-palm processing facilities discard all other edible product, either as animal feed or as compost.

The market for fresh heart in Hawai'i, on the other hand, is eager to exploit all three products. Edible leaf is extremely variable in quantity because it is determined by the expansion of the spear or flag leaf at the moment of harvest. Because of variation in plant height at harvest, unavoidable because of the monthly harvest schedule, height was used as a covariate in these analyses. Moreira & Arkcoll (1988) found a strong relationship between height and individual plant yield, so using this variable as a covariate reduces the within plot variance and permits a better examination of the among progeny variance.

Progeny heart-of-palm yields were significantly different (Table 5.3). Mean heart-of-palm yield was somewhat smaller than the 1.2 t/ha reported by Moreira & Arkcoll (1988), although they harvested taller plants (up to 5 m, but it is unclear how they defined height) than those used here. The Ninole yields are within the range of the Costa Rican commercial yields (0.8-1.0 t/ha) for smaller plants (90-110 cm) that are harvested earlier from densities of 5000 plants/ha with 3-4 stems each (Mora Urpí, pers. comm.), but below the 1.35 t/ha that Mora Urpí (1992a) considers to be attainable.

The heart-of-palm yields reported here (Table 5.3) confirm that pejibaye is well adapted to the Hamakua Coast, although perhaps somewhat less productive than in Latin

America. The edible stem and edible leaf yields enhance the potential of this crop for the fresh market, as it is this extra yield that will guarantee profits to farmers who adopt the crop in Hawai'i.

Table 5.3. Adjusted mean (±SD) potential[a] heart-of-palm, edible stem, edible leaf, and total edible yields up to the end of the first harvest of each pejibaye progeny at Ninole. See Appendix D for mean progeny single-plant weights for each product.

	tons per hectare in one year			
Progeny	Heart	Stem	Leaf	Total
B-0	0.910±0.404	1.957±0.762	0.297±0.178	3.165±1.179
B-1	0.888±0.350	1.887±0.664	0.259±0.168	3.035±1.032
B-2	0.817±0.362	1.998±0.766	0.309±0.174	3.124±1.156
B-3	0.943±0.372	2.022±0.780	0.302±0.187	3.266±1.150
B-5	0.970±0.470	1.989±0.812	0.270±0.156	3.229±1.294
B-8	0.883±0.422	2.034±0.866	0.292±0.164	3.209±1.250
B-9	0.887±0.301	2.114±0.748	0.265±0.163	3.266±1.064
mean	0.910±0.381	2.034±0.754	0.292±0.178	3.236±1.129

[a] assuming 5000 plants/ha and all plants harvested

The cumulative percentage of stems harvested at Ninole and Poamoho was similar (Figures 5.7 and 5.8, respectively), even though Poamoho had some management difficulties during establishment. This suggests that the North Shore of Oahu may be as good a site for pejibaye as the Hamakua Coast of the Island of Hawai'i. Water is the critical variable on the North Shore, but sunlight is more abundant and allows faster growth when water is not limiting. Growth and harvest at Waiakea was much slower (Figure 5.9). This was due to occasional droughts (5-7 days without rain at Waiakea is a drought, because the stony muck has little or no water holding capacity) and, perhaps, less sunlight, since it rains much more.

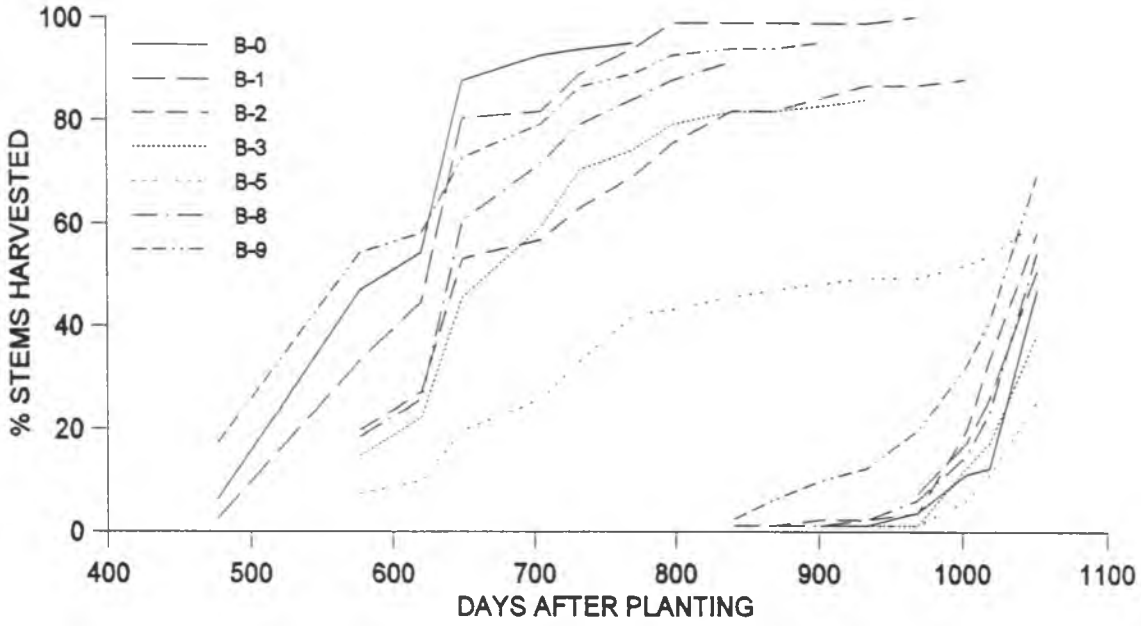

Figure 5.7. Cumulative percentage of pejibaye stems harvested at Ninole throughout the experimental period, with the beginning of the second harvest on the right side.

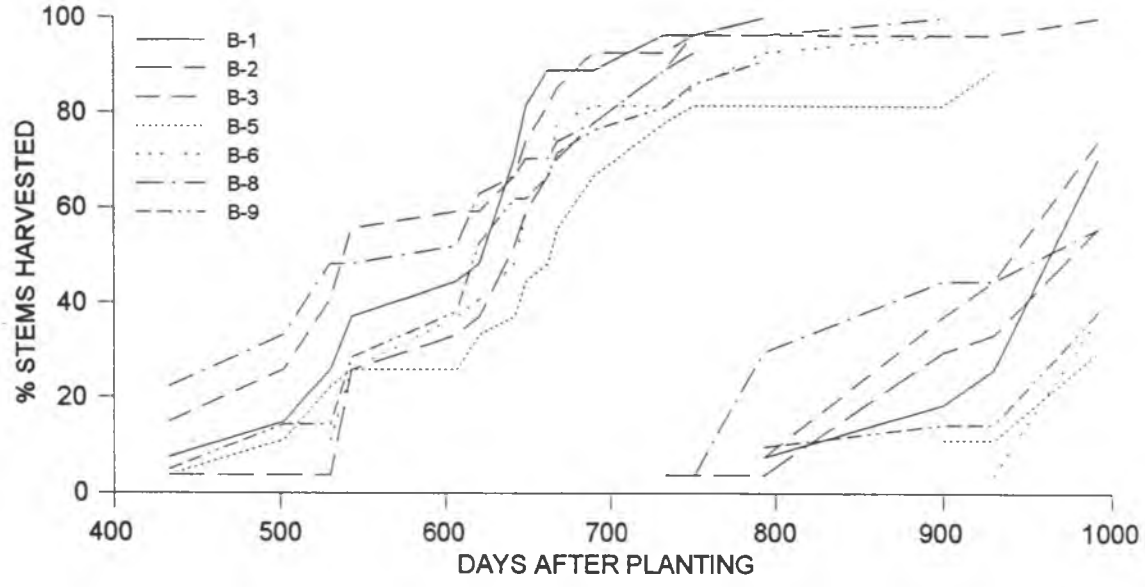

Figure 5.8. Cumulative percentage of pejibaye stems harvested at Poamoho Experiment Station throughout the experimental period, with the beginning of the second harvest on the right side.

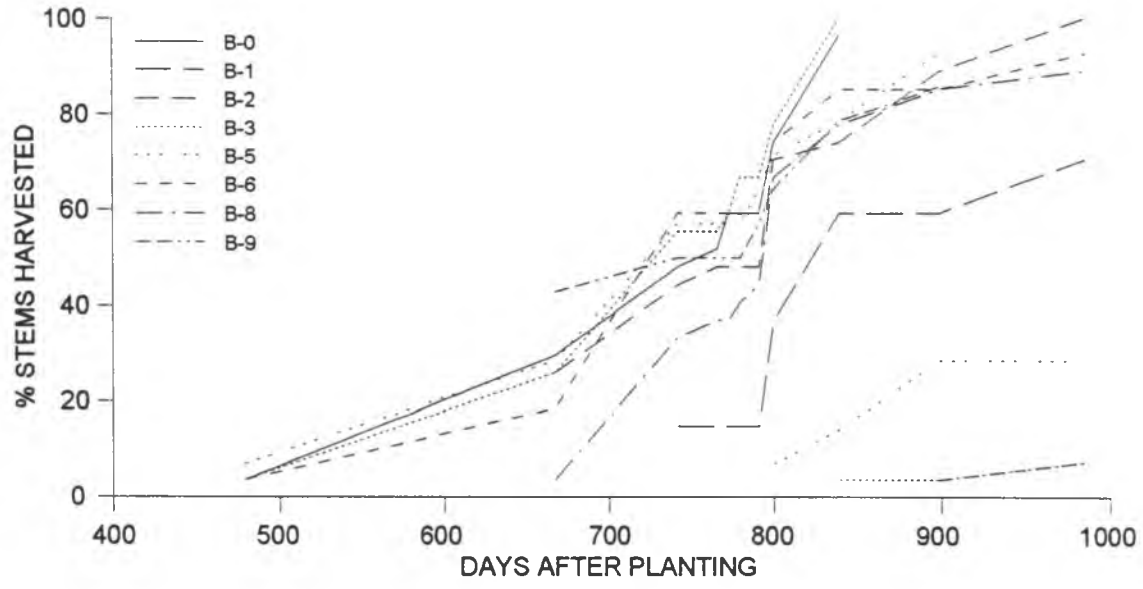

Figure 5.9. Cumulative percentage of pejibaye stems harvested at Waiakea Experiment Station throughout the experimental period, with the beginning of the second harvest in the lower right corner.

5.5. Growth to First Harvest: Uniform Development Phases

There were significant differences in all growth parameters between nursery transplant and the first field evaluation at 178 days in the field (Table 5.4). This interval is the bifid eophyll establishment sub-phase for the slower progenies (B-2, B-3, B-5, B-8) and extends into the beginning of the pinnate establishment sub-phase for the faster progenies (B-0, B-1, B-9). Growth rates in the nursery and during the first six months in the field were very highly correlated with CGR, suggesting that this period is critical in pejibaye, just as it is in coconut (Liyanage 1967). If good nursery practice is used, selection can probably be practiced at the time of field planting and could provide significant advantages if sufficient planting material is available.

Table 5.4. Mean pejibaye progeny crop growth rates (CGR) for the period from nursery transplant to 178 days in the field at Ninole, with mean relative growth rates (RGR), unit leaf rates (E_A) and leaf area ratios (LAR), and progeny mean correlation coefficients between the CGR's and the RGR's, E_A's, and LAR's.

Progeny	CGR (kg/ha/yr)	RGR (g/kg/day)	E_A (g/m^2/day)	LAR (m^2/kg)
B-0	384	15.66	2.00	12.28
B-1	378	15.70	2.03	12.24
B-2	147	12.18	1.36	13.04
B-3	142	12.45	1.40	12.96
B-5	85	10.45	1.09	13.49
B-8	108	12.30	1.39	12.85
B-9	400	14.85	1.82	12.56
mean	235	13.37	1.58	12.77
SD	144	2.03	0.36	0.45
r w/CGR		0.96	0.95	-0.89

Critical values of r are: 0.7545, 0.8745, and 0.9507, for p = 0.05, 0.01, and 0.001 respectively.

The duration of growth from field planting to first harvest was different for each progeny. This period is approximately equivalent to the pinnate-leaved establishment phase (Chapter 4), but not precisely, because each progeny was at a slightly different position in the phase at planting out. The time from field planting to first harvest can be equated with earliness or precocity, so that the vigorous progenies were early while the slow growing progeny was late. The cumulative percentage of plants harvested at each site highlights the earliness of each progeny there (Figures 5.7, 5.8, 5.9).

The harvest at Ninole was interrupted by foraging feral pigs (*Sus scrofa*, Suidae) during March 1994 (at 620 days). While the pigs concentrated on the Yurimaguas population, which they seemed to prefer, the harvest height was reduced from 130 to 110 cm for the Benjamin Constant population to salvage data before the pigs destroyed the

plantation; this is the reason for the abrupt increase in cumulative percentage of stems harvested at that time (Figure 5.7). Twelve percent of the plants in the Benjamin Constant population were destroyed by pigs. The pigs did more damage to the late progeny (B-5) than to the others (Table 5.5), suggesting that this progeny had some characteristic similar to the Yurimaguas population, in which 50% of the plants were also damaged.

Table 5.5. Percentage of plants in each progeny damaged by pigs in March 1994.

Progeny:	B-0	B-1	B-2	B-3	B-5	B-8	B-9
% damaged	4.9	1.2	11.1	16.0	45.7	7.4	4.9

The pig damage in March 1994 also complicates the interpretation of the growth parameters calculated for progenies B-2, B-3 and B-5. Nonetheless, growth and harvest data before the pig damage confirm that these progenies were slower growing than the vigorous progenies. In the estimation of growth parameters below, only undamaged plants were considered and I assume that the pigs damaged plants randomly, rather than concentrating on fast or slow growing individuals within each progeny. Since the pigs obviously had preferences for progenies, however, this assumption may not be valid.

At harvest there were significant differences among progenies for number of offshoots and leaves and for whole plant leaf area, but not for above ground biomass (Table 5.6). Progeny B-9 was different from the others, maintaining one more leaf at harvest. Maintenance of more leaves probably explain this progeny's faster growth and earlier harvest. There were significant differences among the progeny means for LAR,

with progeny B-9 having the highest LAR. The variation in LAR was relatively small, which is probably due to the plants being harvested at the same ontogenetic stage.

Table 5.6. Pejibaye progeny mean plant dimensions and leaf area ratio (LAR) at first harvest at Ninole.

Progeny	offshoot number	leaf number	leaf area (m^2)	biomass (kg)	LAR (m^2/kg)
B-0	7.4	6.3	7.97	2.12	3.77
B-1	9.9	6.1	7.95	2.13	3.74
B-2	8.7	6.4	8.26	2.20	3.77
B-3	6.9	6.0	7.97	2.16	3.70
B-5	9.3	6.2	7.89	2.10	3.78
B-8	6.6	6.2	7.64	2.02	3.80
B-9	7.1	7.4	8.83	2.27	3.90
mean	8.0	6.4	8.07	2.14	3.78
SD	1.3	0.5	0.38	0.08	0.06

A priori a high negative correlation was expected between earliness and RGR, since the higher the RGR the earlier the progeny. The correlations found suggest a tendency in the correct direction for the growth rates calculated between field planting and harvest (Table 5.7). The probable reason for the lack of significant correlations is that the more vigorous progenies were larger when field planted and this growth in the nursery is not accounted for in the time interval examined here.

The significant correlation between LAR and earliness suggests its use as a selection criterion in pejibaye (Table 5.7). Hardon et al. (1972) and Breure & Corley (1983) found that LAR was correlated with fruit yield in African oil palm and that there was significant genetic variation for this trait. They used a uniform time period to evaluate LAR,

however, rather than evaluating plants that had attained a uniform developmental stage. The magnitude of the phenotypic variation of these growth parameters is examined in Chapter 6.

Table 5.7. Pejibaye progeny means at Ninole for days to harvest (DTH), crop growth rate (CGR), relative growth rate (RGR), unit leaf rate (E_A) and leaf area ratio (LAR) that may explain earliness for the period from field planting to first harvest, and progeny mean correlation coefficients between each rate or ratio and earliness.

Progeny	DTH (days)	CGR (t/ha/yr)	RGR (g/kg/day)	E_A (g/m^2/day)	LAR (m^2/kg)
B-0	610	8.8	7.31	1.63	5.3
B-1	642	8.0	6.89	1.56	5.3
B-2	692	7.9	9.04	2.04	6.0
B-3	684	7.9	8.92	2.04	5.8
B-5	726	7.0	8.87	2.00	6.0
B-8	666	7.6	9.44	2.12	5.9
B-9	611	9.4	8.10	1.77	5.6
mean	662	8.1	8.37	1.88	5.7
SD	43	0.8	0.96	0.22	0.3
r w/DTH		-0.90	0.67	0.72	0.82

Critical values of r are: 0.7545, 0.8745, 0.9507, for p = 0.05, 0.01, 0.001, respectively.

When these same growth parameters are evaluated over the life span of the plants (Table 5.8), the high negative correlation between earliness and RGR is found. The very high negative correlation between earliness and E_A suggests that this parameter is more important than LAR in these progenies, but the relative importance of E_A and LAR varies in different progenies.

There were significant differences among progenies for all growth rates between nursery transplant and first harvest. The two earliest progenies, B-0 and B-9 appear to

grow rapidly for different reasons. Progeny B-0 has the highest E_A but only an average LAR. This suggests that B-0 may have greater photosynthetic efficiency. Progeny B-9, on the other hand, has both a high E_A (although lower than B-0) and the highest LAR. This suggests that B-9 has a somewhat lower photosynthetic efficiency, but partitions more of its photoassimilates to leaf area.

Table 5.8. Pejibaye progeny means at Ninole for days to harvest (DTH), crop growth rate (CGR), relative growth rate (RGR), unit leaf rate (E_A), and leaf area ratio (LAR) that may explain earliness for the period from nursery transplant to first harvest, and progeny mean correlation coefficients between each rate or ratio and earliness.

Progeny	DTH (days)	CGR (t/ha/yr)	RGR (g/kg/day)	E_A (g/m^2/day)	LAR (m^2/kg)
B-0	898	4.37	11.71	2.66	10.71
B-1	932	4.18	11.33	2.59	10.69
B-2	982	4.12	10.82	2.45	10.71
B-3	972	4.13	10.87	2.51	10.67
B-5	1027	3.80	10.31	2.33	10.71
B-8	954	3.92	11.00	2.47	10.72
B-9	899	4.64	11.85	2.61	10.77
mean	952	4.17	11.13	2.52	10.71
SD	47	0.28	0.54	0.11	0.03
r w/DTH		-0.85	-0.99	-0.96	-0.41

Critical values of r are: 0.7545, 0.8745, 0.9507, for p = 0.05, 0.01, 0.001, respectively.

The fact that the expected negative correlation only appeared when the entire life span of the plant was included suggests that the early establishment phase is extremely important. This, in turn, suggests that early growth can be used to identify outstanding progenies and individuals, as is true with coconut (Liyanage 1967). As Tan & Hardon (1976) point out, however, the major question refers to the heritabilities of the juvenile

traits correlated with later growth. Due to the delay in planting and guaranteeing field establishment in this project, this early growth was not examined in sufficient detail.

5.6. Growth Before First Harvest: Uniform Time Intervals

The shape of the progeny growth curves at Ninole during the establishment phase (Figure 5.10) is approximately exponential, as was also observed for the growth of African oil palm during its establishment phase (Henry (1958) cited by Corley (1976a)). Exponential growth commonly occurs during vegetative growth after the seedling has ceased to be dependent on seed reserves until limited by some stress, including competition (Causton & Venus 1981). For the vigorous progenies, the growth curves in Figure 5.10 cover the majority of the establishment phase, while for the intermediate and slow progenies the latter part of the establishment phase is not presented.

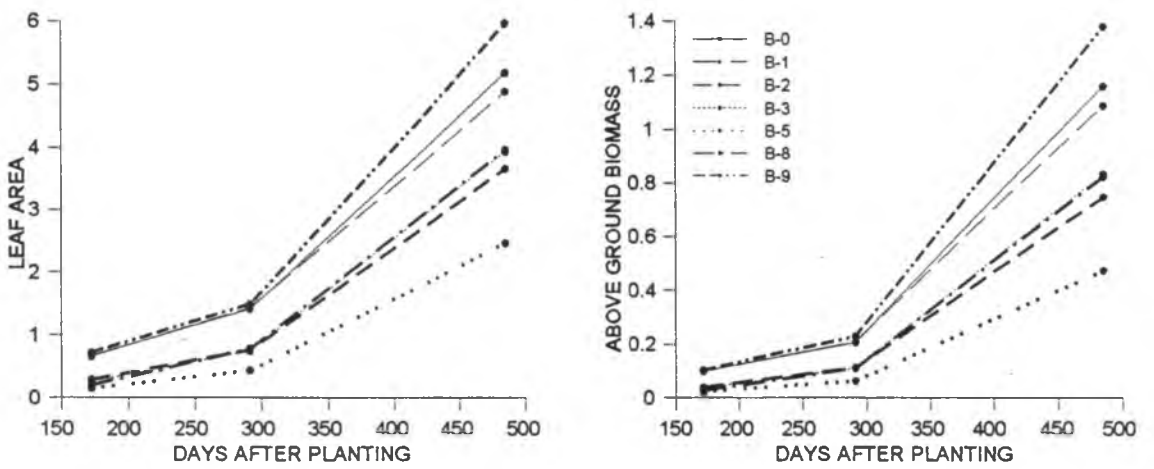

Figure 5.10. Mean whole plant leaf area (m^2; Left) and above ground biomass (kg; Right) of seven pejibaye progenies planted at Ninole from the first to the third evaluations.

There were significant differences among progenies for whole plant leaf area and above ground biomass at each sampling time. At 172 and 293 days after planting, the three vigorous progenies were different from the others. At 486 days, the most vigorous progeny (B-9) was different from the intermediate group; the other vigorous progenies (B-0, B-1) were not different from the intermediate group, but were different from the slowest progeny.

The progeny mean LAR's generally decreased over time (Figure 5.11), and similar results have been observed in African oil palm (Corley & Gray 1976a) and other plants in general (Causton & Venus 1981). There were significant differences among the progeny LAR means at all sampling times. Five of the seven progenies had similar LAR curves. The LAR's of progenies B-0 and B-1, however, were very low at the first sampling and increased at the second, before decreasing at the third. Since both of these progenies are quite vigorous, they were expected to be decreasing at the second measurement in the same manner as progeny B-9, the third vigorous progeny. A careful reexamination of the data files at both dates did not turn up any errors in digitation or calculation, so these anomalous results remain unexplained.

When exponential growth occurs during a given time period a constant RGR is implied (Causton & Venus 1981), although constant RGR's are rarely observed in practice (Corley & Gray 1976a) because climatic and management variation during a given time interval can cause growth to increase or decrease rapidly. Three of the seven progenies exhibited nearly constant RGR's during the two intervals evaluated before first

harvest (Table 5.8), while three progenies grew more rapidly and one more slowly in the second interval than the first.

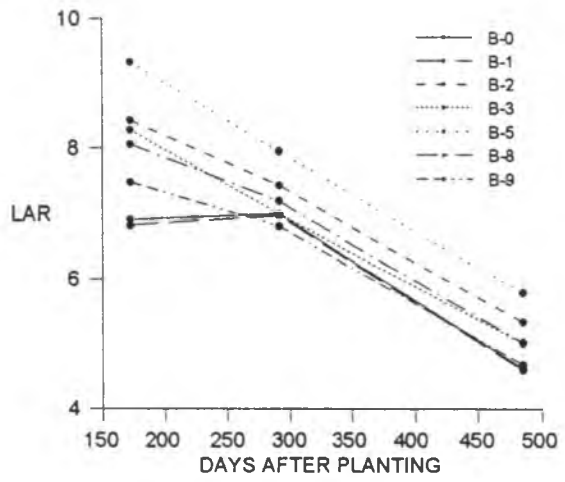

Figure 5.11. Leaf area ratios (LAR) of seven pejibaye progenies at Ninole at 172, 291 and 485 days after field planting.

Unit leaf rates increased in all progenies from the first to the second interval before first harvest (Table 5.9). The E_A of the more vigorous progenies increased by 30 to 40% from one interval to the next. Interestingly, the E_A of the least vigorous progeny, B-5, increased by 25% and the RGR by 10%. The slower growth of the majority of the plants in this progeny may be due to unfavorable partitioning between the main stem and its offshoots, greater partitioning to it roots, or possibly to greater respiration losses.

The correlation between the CGR's and the RGR's and E_A's was negative, rather than positive, which suggests that either the progenies were in different development phases or a critical interval was lacking. During the development of a plant, RGR, and its components, E_A and LAR, decrease gradually from a maximum attained during the seedling phase (Causton & Venus 1981). The decrease in growth rates is due to an

increase in non-photosynthesizing biomass that requires resources for its maintenance. The low RGR's of progenies B-0, B-1 and B-9, therefore, are the result of their larger size during the intervals. The opposite holds true for the other four progenies. The CGR's, on the other hand, take into account the size of the plants at the beginning and end of the interval. The variation in the correlations for different intervals suggests that the vigorous, intermediate and slow progenies were at different ontogenetic stages (Evans' (1972) terminology, cited by Coleman et al. (1994)) during each period. The missing interval is the bifid eophyll sub-phase, especially of progenies B-0, B-1 and B-9.

Table 5.9. Pejibaye mean progeny crop growth rates (CGR) during the year before the first harvest, and relative growth rates (RGR) and unit leaf rates (E_A) during two intervals before the first harvest at Ninole, and progeny mean correlation coefficients between the CGR's and the RGR's and E_A's.

	CGR[a]	RGR[b]		%	E_A[c]		%
Progeny	172-485[d]	172-291	291-485	diff.	172-291	291-485	diff.
B-0	6.21	6.83	8.91	23.3	0.98	1.66	41.0
B-1	5.66	6.85	8.63	20.6	1.00	1.61	37.9
B-2	4.04	11.26	11.60	2.9	1.49	1.93	22.8
B-3	4.70	11.85	11.51	-2.9	1.60	2.05	21.9
B-5	2.67	11.28	12.80	11.9	1.46	1.95	25.1
B-8	4.71	13.35	11.14	-19.8	1.82	1.96	7.1
B-9	7.45	9.14	9.68	5.6	1.28	1.81	29.3
mean	5.06	10.08	10.61		1.38	1.85	
SD	1.55	2.53	1.56		0.31	0.17	
r w/CGR		-0.58	-0.84		-0.51	-0.58	

Critical values of r are: 0.7545, 0.8745, for p = 0.05, 0.01, respectively.

[a] CGR in t/ha/year
[b] RGR in g/kg/day
[c] E_A in $g/m^2/day$
[d] Days after planting

5.7. Regrowth Between Harvests: Uniform Time Intervals

The data set used to evaluate growth from the first to the second harvest is much less representative of the population as a whole at Ninole than that used to evaluate growth from field planting to the first harvest. The reason is that only 60% of the plants from the first cycle were included and the 40% excluded were not chosen randomly. All plants that had not been harvested before 702 days (including those damaged by pigs in March 1994) and all that were harvested during the 702-998 day interval were excluded. Thus, exceptionally slow and very fast growing plants were not included, resulting in a significant reduction in variation within and among the progenies. This was done in order to compare plants that were present during the entire interval.

Management of the plants was also different during the period under consideration. The most important variable was offshoot thinning: all clumps were thinned to two offshoots just before the first measurement during the regrowth period. Because of the competition among offshoots within a clump before thinning, most offshoots had only two or three leaves after thinning. On average, the smaller offshoot was 80% of the size of the larger offshoot. The second important management variable was fertilization: one month after thinning, the quantity of fertilizer applied was doubled, by applying the same amount at shorter intervals, i.e. 100 g/plant every two months instead of every four months. This change in fertilizer regime was necessary because there were then twice as many plants in the field as during the first harvest cycle.

Growth after thinning was much more vigorous than during the first harvest cycle (Figure 5.12; Table 5.10). The major factor favoring rapid growth after thinning was that

the offshoots were already well established, with extensive roots systems that had probably already been infected with mycorrhizae. Consequently, the plants could invest immediately in aerial growth, rather than build a root system.

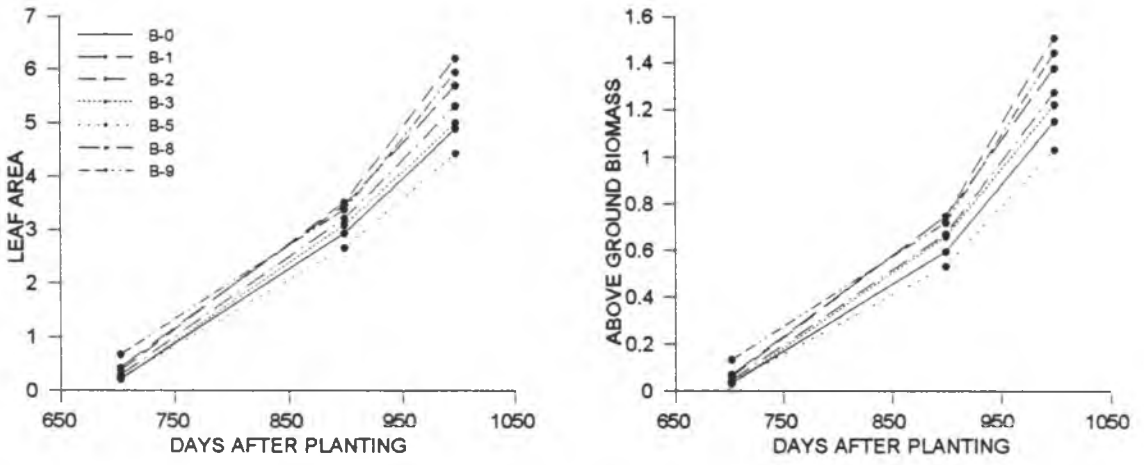

Figure 5.12. Mean pejibaye whole plant leaf area (m^2; Left) and above ground biomass (kg; Right) of each progeny at Ninole from the fourth to the sixth evaluations, without including plants that were harvested during the period.

There were highly significant differences in mean progeny whole plant leaf areas and above ground biomass at all three measurement dates, but not in mean CGR's, RGR's and E_A's (Table 5.10) and only at 899 days for LAR's (Figure 5.13). This suggests that the reduced data set used for this evaluation grew much more uniformly over the period than was the case in the first harvest cycle. The 40% of plants excluded were precisely the ones that provided greatest variation and differentiation among progenies during the first harvest cycle.

All progenies grew well during the first interval, but much less well during the second, as evidenced by enormous declines in RGR and E_A. These declines are explainable by climatic variation: in November 1994, just before the fifth evaluation, it

rained heavily everyday for 30 days (Chapter 3, Figure 3.1). Not even the Guinea grass and other weeds grew during this period. In February, the most recent *El Niño* event caused a moderate drought and rainfall at Ninole dropped to only 2.3 inches.

Table 5.10. Mean pejibaye crop growth rate (CGR) during the period between harvests, relative growth rate (RGR) and unit leaf rate (E_A) during two intervals before the second harvest at Ninole, and progeny mean correlation coefficients between the CGR's and the RGR's and E_A's.

	CGR[a]	RGR[b]		%	E_A[c]		%
Progeny	702-998[d]	702-899	899-998	diff.	702-899	899-998	diff.
B-0	6.95	20.30	6.86	-195.9	3.59	1.46	-145.9
B-1	7.96	18.32	6.18	-196.4	3.43	1.40	-145.0
B-2	8.92	18.52	6.91	-168.0	3.42	1.56	-119.2
B-3	7.34	21.51	6.34	-239.3	3.84	1.38	-178.3
B-5	6.31	18.38	6.68	-175.1	3.15	1.40	-125.0
B-8	7.46	19.05	6.41	-197.2	3.43	1.41	-143.3
B-9	8.15	16.78	7.06	-137.7	3.08	1.57	-96.2
mean	7.58	18.98	6.63		3.42	1.45	
SD	0.85	1.53	0.33		0.26	0.08	
r w/CGR		-0.36	0.20		-0.04	0.68	

Critical values of r are: 0.7545, 0.8745, 0.9507, for p = 0.05, 0.01, 0.001, respectively.
[a] CGR in t/ha/year
[b] RGR in g/kg/day
[c] E_A in $g/m^2/day$
[d] Days after planting

The relative order of growth superiority during the second harvest cycle was different from that in the first. This may be due partially to the size of the plants at thinning (Table 5.11). The change in order is also evident in the number of plants harvested from the precocious progenies during the period (Figure 5.7).

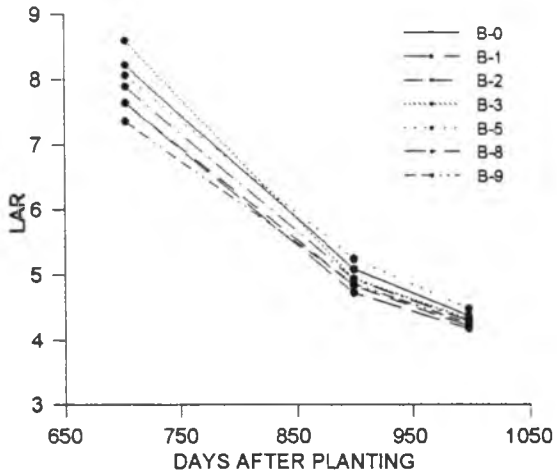

Figure 5.13. Leaf area ratios (LAR) of seven pejibaye progenies at Ninole at 702, 899 and 998 days after planting.

Table 5.11. Mean height of the largest offshoot in each pejibaye progeny immediately after thinning the clump.

Progeny:	B-0	B-1	B-2	B-3	B-5	B-8	B-9	$LSD_{(0.05)}$
height (m)	0.19	0.23	0.23	0.19	0.20	0.21	0.29	0.067

Progeny B-2, previously only an intermediate progeny, was the fastest growing progeny. Curiously, it continued to be intermediate in E_A and LAR. During the climatic stress interval, however, it had a high E_A, suggesting that, like B-9, it is more stable in adverse situations.

Progeny B-9 continued to present superior growth and earliness. During the first time interval, it was inferior to the other progenies in E_A (Table 5.10). During the second interval, however, it was the best, which suggests that it is more stable in adverse situations than most of the other progenies.

Progeny B-0, previously an outstanding progeny, was the second slowest progeny during the second cycle. This change is difficult to explain because it continued to have a

high RGR and E_A, and appears to have handled the climatic stress during the period reasonably well. In Figure 5.7, however, this progeny increased more rapidly than others in the final harvest, suggesting that its reduced size during the uniform time intervals was due principally to the size of the offshoots at pruning (Table 5.11).

As during the first harvest cycle, the correlations between CGR and RGR and E_A were not significant. This suggests that the critical growth period for determining earliness was not included in the interval evaluated between harvests. Unlike the first harvest cycle, it is impractical to extend the growth estimates backward in time, because each progeny was different in earliness of offshoot initiation and how resources were partitioned to these. Comparing Tables 5.6 and 5.11 provides an idea of how each progeny partitions resources to its offshoots. Progenies B-1 and B-5 had the greatest number of offshoots at harvest and both had relatively small offshoots just after clump thinning. Progeny B-9, on the other hand, had fewer offshoots at harvest, but significantly larger ones. This combination may explain B-9's overall superiority during the second harvest cycle, especially when combined with its rapid growth throughout, higher LAR, and apparent tolerance of environmental stress.

5.8. Correlations Between Germination and Growth

Correlations were examined between progeny mean germination variables (Table 5.1) and growth parameters from nursery transplant to first evaluation (Table 5.4), from first evaluation to first harvest (Table 5.7), from nursery transplant to first harvest (Table 5.8), and from first to third evaluations (Table 5.9). The set that produced the most significant

correlations was between nursery transplant and first harvest (Table 5.8), although those from nursery transplant to first evaluation were almost as high (data not shown).

The only significant correlation, however, is between leaf length at transplant and mean LAR during the interval, which suggests that LAR could be selected for within a few months after germination. A nearly significant correlation is that between bifid eophyll number at transplant and days to harvest. The existence of these correlations suggests that further study is warranted. There are many correlations that do not appear to make any sense at all, however, which reenforces the need for more care in managing the seed before starting an experiment.

Table 5.12. Pejibaye progeny mean correlation coefficients between germination variables (Table 5.1) and growth parameters estimated from nursery transplant to first harvest (Table 5.8).

	Viability	Seed Wt.	Germ. %	EVI	Bifid #	Leaf L.
DTH	-0.341	-0.290	0.216	0.465	0.636	-0.426
CGR	0.036	-0.208	-0.315	-0.398	-0.562	0.344
RGR	0.292	0.186	-0.260	-0.468	-0.633	0.436
E_A	0.123	0.172	-0.154	-0.387	-0.622	0.215
LAR	0.515	0.046	-0.423	-0.388	-0.198	0.767

The critical value of r is 0.7545, for p = 0.05.

5.9. Conclusions

The germination parameters presented several unexpected correlations that suggested that seed management before sowing was not uniform, which is confirmed by the low germination percentage (53%). This lack of adequate management, in turn, made other conclusions based on germination of dubious value. Nonetheless, the correlation between

the length of the largest bifid eophyll at transplant and leaf area ratio over the period from transplant to harvest suggests that some selection might be usefully practiced early in the nursery period.

There were no density effects, nor effects of position within the plot. This suggests that plot size was too small, that the reduced leaf number reported in Chapter 4 lowered LAI and thus competition, or that these densities did not reduce vegetative growth of the plants, as was also observed in African oil palm at higher than optimum fruiting densities (Corley 1973). The lack of density and position effects allowed the use of all plants for these growth studies and the subsequent genetic analysis (Chapter 6).

Growth in the nursery and the first six months in the field was highly correlated with days to first harvest. This suggests that selection can be practiced at the time of field planting. In this case, plant height and leaf number can be used to identify those plants and progenies with greatest potential for vigorous growth and early harvest.

There was considerable variation in growth and growth parameters during all of the intervals examined, both over uniform time periods and over uniform developmental stages. Uniform time periods presented greater variation among the progenies than uniform developmental stages. This makes biological sense, since, during any given time interval, different progenies (and even different plants within a progeny) are at different developmental stages, which implies different growth rates to attain a given stage at a given time.

Relative growth rate was found to be highly and negatively correlated with days to first harvest, as expected. Less expected was the fact that unit leaf rate explained the

majority of this relationship. Unfortunately, E_A tends to have low heritability because of the numerous environmental factors that influence it (Gupta 1992). Nonetheless, there may be exploitable genetic variation for E_A that can be captured through selection for earliness (days between field planting and first harvest).

There was some variation in the way progenies partitioned photoassimilates. Among the three most vigorous progenies up to first harvest, two progenies (B-0, B-1) had higher E_A with partitioning to biomass in general, while the third progeny (B-9) had only an average E_A but partitioned more to leaf area, resulting in a higher LAR. These two growth strategies are commonly observed in crops and non-crop plants (Causton & Venus 1981). Over the entire experimental period at Ninole, progeny B-9 maintained its rapid growth, which suggests that LAR may be the most important growth parameter in that environment. At Poamoho and Waiakea, however, progeny B-9 presented only average growth rates, which suggests that high LAR may not always be advantageous.

A third way of partitioning was observed for the least precocious progeny, B-5. In the two better environments, Ninole and Poamoho, this progeny consistently performed the worst, except in individual plant heart-of-palm weight. At Waiakea, however, which was the most stressful environment, this progeny performed amongst the best. At Ninole, progeny B-5 was observed to be relatively stable during periods of stress, so that its better performance at Waiakea may be due to this stability of growth. It is possible that this progeny partitions more to root growth, at the expense of shoot growth. It doesn't grow well, but when all other progenies are growing poorly because of environmental stress, B-5 appears to be better.

Potential heart-of-palm yields at Ninole were acceptable, with about 900 kg/ha at 5000 plants/ha for the first harvest. Actual yields, due to pig damage and the occasional extremely slow growing plant, were somewhat less. Nonetheless, these yields show that pejibaye is well adapted to the Hamakua Coast of the Island of Hawai'i. Regrowth after first harvest was strong and, with two stems per clump, 18 month yields were about 1.4 t/ha.

In general, the use of classical growth analysis methods allowed the identification of differential partitioning of photoassimilates, as well as considerable genetic variation, both of which may be amenable to selection in an improvement program.

Chapter 6. Genetic Analysis

6.1. Introduction

Plant breeding relies upon a secure knowledge of the desires of the farmer and consumer, the agricultural system to be used, and the essential genetic features of the crop to be improved (Simmonds 1979). In this chapter, the genetic variability of several traits of interest to farmers and consumers is examined, focussing on that portion of the genetic variability that is amenable to selection, i.e., the additive genetic variance.

The only previous experimental work published on the genetics of pejibaye examined spines on the petiole/rachis, leaflet mid-rib and leaflet edge (Chávez Flores et al. 1990). These authors examined progenies sampled from the Yurimaguas population of the Pampa Hermosa landrace and found that the most important of these three traits, spines on the petiole, had a narrow-sense heritability of 0.36. The occurrence of spines is primitive in *Bactris* (Uhl & Dransfield 1987), which led Mora Urpí (personal communication) to hypothesize that their presence is dominant to their absence. Consequently, a spineless plant would be a homozygous recessive and a degree of inbreeding might be expected in populations with a high level of spinelessness.

Clement (1988) suggested that the domestication process in pejibaye may have caused a significant level of inbreeding, because of small population sizes, the likelihood of related individuals in a given swidden, and a restricted exchange network for each village's germplasm base. Plants of the Benjamin Constant pejibaye population set significant quantities of fruit after self-pollination (Clement & Arkcoll 1984), even though Mora Urpí (1981) postulated the existence of a self-incompatibility mechanism in

the species. Inbreeding can slow the response to selection because there is less additive genetic variance present than would be the case in a more heterozygous population (Falconer 1981).

While little genetic analysis has been done with pejibaye, quite a lot has been done with African oil palm (*Elaeis guineensis* Jacq.). As in other areas, oil palm is a useful guide to pejibaye. Hardon (1970) showed that unintentional inbreeding slowed the response to selection in oil palm. The recurrent recombinant improvement strategy used by the French breeders (Meunier & Gascon 1972) was designed to avoid inbreeding and exploit heterotic effects found in some wide hybridizations with African germplasm.

Hardon, Corley and their colleagues studied the genetics of vegetative growth in oil palm to permit more efficient selection for partitioning ratios and competition tolerance (Corley et al. 1971, Hardon et al. 1972, Breure & Corley 1983). They found moderate heritabilities for several vegetative traits and growth parameters, several of which are the object of this study with pejibaye. While heritability estimates can not be used directly with other germplasm or in other environments (Simmonds 1979), they provide a guide of what to expect. A selection of the oil palm heritability estimates was presented in Chapter 2, Table 2.3.

Simmonds (1979) and Gupta (1992) emphasize that there has been little response to selection for the most basic growth parameters, e.g., relative growth rate or unit leaf rate. While it is certainly true that there are significant environmental effects on these traits, it is not completely clear why response has been so limited. One possible factor, at least in perennials, is the way in which these traits are studied. Most research is done over

uniform time periods, a year or six months, for example, which may mix information from different developmental stages (Coleman et al. 1994). Chapter 5 contrasted the calculation of growth parameters over uniform developmental periods and uniform time intervals. Days to harvest was found to be highly correlated with parameters from uniform developmental periods. The implications of this contrast are examined in the current chapter.

Most genetic analysis in crops is based on large numbers of individuals of a reasonably large number of progenies replicated a large number of times, either in space or in time or both (cf. Simmonds 1979, Nyquist 1991). The current trial is limited in all of the above, since pejibaye is a new crop in Hawai'i and only limited amounts of germplasm and resources were available for experimentation. Environments were selected based upon current and future trends in Hawai'i's agricultural economy, rather than on suitability for pejibaye, since this was unknown at any rate.

Simmonds (1979) emphasizes the importance of border rows to eliminate border effects. If the agricultural system used is based on rows, however, it makes sense to include edge rows in the sample used for genetic analysis (J.L. Brewbaker, personal communication). In Hawai'i, pejibaye for heart-of-palm is most likely to be grown in double or triple rows, so that weed management, fertilization and harvesting can be mechanized to the maximum extent possible. Edge effects on individual plant dimensions were found to be statistically insignificant in this trial (Chapter 5). Thus, the case is made to include the border rows in this analysis to provide more statistical power to compensate in part for the limitations in progenies and environments. This is especially

important given the small plot size used (9 plants). With these departures from traditional practice recognized beforehand, the results can be treated with the caution they require.

Following practice in genetic research on oil palm (e.g., Hardon et al. (1972)), the additive genetic variances and narrow-sense heritabilities estimated here are for individuals within families, rather than family means. The reason is that only a very limited set of outstanding plants is likely to be used in a future improvement program in Hawai'i and, in order to maximize heterotic potential, they should be as distantly related as possible. As in oil palm, these 'elite' individuals will be crossed to produce new progenies for testing.

In any improvement program, a limited set of primary objectives is essential to guide the breeder (Simmonds 1979). In pejibaye for heart-of-palm, the traits of interest are spinelessness, rapid growth, sufficient suckering, and no acridity. In this chapter, the genetic structure of the Benjamin Constant population is examined. The absolute magnitude of the phenotypic variance and its coefficient of variation are presented for each trait in each experimental environment, and the proportion due to additive genetic variance is estimated, with the assumption that these are samples from a outbreeding, panmictic population. The response to selection for the most important trait in the long term, rapid growth, is examined in detail.

6.2. Spines

The elimination of spines is certainly a high priority for selection of pejibaye in Hawai'i. The germplasm introduced from Brazil was selected exclusively from plants

without spines on the petiole/rachis and stem. Since this germplasm was open-pollinated and there were spiny palms nearby, however, some spininess was expected. Only one progeny (B-0) presented 100% spineless-petioled plants (Table 6.1). Mean spininess (based on the ordinal scale: 0, 1-9 (Chávez Flores et al. 1990)) was low in all progenies because of the high frequency of spinelessness.

Table 6.1. Frequency (% of plants) of spininess (present, absent), mean spininess (0, 1-9), and frequency of different types of developmental change in spininess for plants with spines (spineless plants were constantly spineless), for all field-grown plants of the nine Benjamin Constant pejibaye progenies in Hawai'i.

		Spines		Mean	Changes in Spininess[a]		
Progeny	n	Present	Absent	Spininess	Constant	Slow	Rapid
B-0	108	0	100	0	0	0	0
B-1	135	22.2	77.8	1.31	13.3	5.9	3.0
B-2	135	26.7	73.3	1.56	11.1	14.1	1.5
B-3	135	29.6	70.4	1.65	9.6	11.9	8.1
B-5	126	29.4	70.6	1.81	16.7	11.9	0.8
B-6	108	28.7	71.3	1.89	14.8	13.0	0.9
B-7	27	22.2	77.8	1.51	11.1	7.4	3.7
B-8	135	25.2	74.8	1.52	14.1	8.1	3.0
B-9	120	38.3	61.7	2.56	14.2	22.5	1.7

[a] Developmental changes in spininess, as described in Chapter 4, section 4.3.1.

Most of the progenies were similar with respect to the frequencies of plants presenting contrasting developmental changes in presence of spines. Only progeny B-0 was clearly different (Table 6.1). Progeny B-3, however, presented approximately equal numbers of spiny plants in each developmental group. If the slow and rapid change classes are merely slightly different manifestations of the same gene or gene block, the frequency of 'constant' versus 'change' classes was approximately equal, except for

progenies B-3 and B-9. In these two progenies, twice as many spiny plants presented reduced spininess during ontogeny as remained constant.

The additive genetic variances (V_P * h^2) for spines on the petiole/rachis and the corresponding heritability estimates were generally quite low (Table 6.2). Chávez Flores et al. (1990) obtained an estimate of $h^2 = 0.36$ in the Yurimaguas population for this trait. Only the sample at Waiakea was similar. The estimate of zero additive genetic variance in the full combined analysis is an approximation, since the analysis yielded a negative estimate for the progeny effect. One of the main reasons for this estimate is the small number of very similar progenies used. Progenies B-0 (completely spineless) and B-9 (the most spiny) were the most different and were not used in the combined analysis because of their absence at one site.

Table 6.2. The genetics of pejibaye petiole spines and developmental changes in spininess in the Benjamin Constant population in Hawai'i. Phenotypic variances (V_P), coefficients of variation (CV_P), and narrow-sense heritabilities (h^2) (assuming no inbreeding (F = 0)) for each trait at each site and over combinations of sites. Because inbreeding is likely (Chapter 7, section 7.3), $h^2_i = h^2$ (1-F), and F could = 0.50.

	Spininess			Developmental Changes		
Site	V_P	CV_p	h^2	V_P	CV_p	h^2
Ninole	0.516	52.9	0.27	0.0154	8.5	0.17
Poamoho	0.601	50.0	0.14	0.0338	12.2	0.22
Waiakea	0.509	48.9	0.40	0.0069	5.8	0.04
N & P	0.557	51.4	0.07	0.0239	10.4	0.08
N & W	0.430	48.3	0.15	0.0110	7.3	0
N & P & W	0.510	49.8	0	0.0197	9.6	0.04

There are at least two possible explanations for the low additive genetic variance estimates: 1) the history of the Benjamin Constant population sample (see Chapter 3); and 2) the clear environmental differences among the experimental sites.

The genetic variability for spininess in this sample must have been severely reduced by the series of selections against spines practiced during the formation of the several seedling orchards. Additionally, this sampling strategy (small samples of possibly closely related germplasm - all spineless) in successive seedling orchards, would increase the likelyhood of inbreeding (through sib-mating) at each step.

Another reason for the low estimates was the large interaction variances. Since Chávez Flores et al. (1990) found a relatively low narrow-sense heritability of 0.36, there is the possibility of large environmental influences on this trait. The Waiakea site appeared to favor spininess, and the greater expression of spininess there may be the reason for the larger additive genetic variance.

The genetic variances and heritabilities for the developmental changes in spininess were even lower than for the presence of spines on the petiole/rachis (Table 6.2). The same reasons just mentioned probably apply. Using the developmental changes in spininess of plants at Ninole as the standard, plants at Poamoho were more changeable (higher frequency of plants with slow and rapid developmental changes), while Waiakea was more constantly spiny. Waiakea was the most stressful environment during this experiment, since even a week without rain resulted in drought conditions because of the lack of water holding capacity in the stony muck. This periodic stress may have been sufficient to enhance spininess at that site. Further study of this apparent developmental

change in pejibaye may provide some insight into its evolutionary history, but is not worth pursuing in Hawai'i, where only spineless plants will be used in the future.

6.3. Growth Parameters Over Different Intervals

The genetics of the major growth parameters, i.e., relative growth rate (RGR), unit leaf rate (E_A), and leaf area ratio (LAR), can be examined over different intervals in the crop growth cycle (Chapter 5, sections 5.5 and 5.6). Each interval has different developmental, genetic and practical implications. In order to select for early harvest, rapid growth is desired over a uniform developmental period. Shorter periods may have important correlations with early harvest, especially the nursery period and establishment phase. Examining a uniform time period is often easiest with an established plantation, however, and is most often practiced with perennial crops. These three possible intervals are examined below.

The growth period from nursery transplant to harvest was identified as extremely important because of its very high correlation with early harvest (Chapter 5, section 5.5). This variable time interval, different for each progeny, covers a uniform developmental period - seedling to early adult vegetative phase. Perhaps because the plants were phenotypically very similar at both ends of this period, the coefficient of phenotypic variation was extremely low (Table 6.3), especially for LAR. The low CV_p for RGR and E_A was more surprising, however, because both of these growth parameters include time in their estimation.

Table 6.3. The genetics of pejibaye growth over a uniform developmental phase. Phenotypic variances (V_P), coefficients of variation (CV_P), and narrow-sense heritabilities (h^2) (assuming no inbreeding (F = 0)) for relative growth rate (RGR), unit leaf rate (E_A) and leaf area ratio (LAR), were calculated from nursery transplant to first harvest, at each site and over combinations of sites. Because inbreeding is likely (Chapter 7, section 7.3), $h^2_i = h^2 (1-F)$, and F could = 0.50.

	RGR			E_A			LAR		
Site	V_P	CV_p	h^2	V_P	CV_p	h^2	V_P	CV_p	h^2
Ninole (all)	1.20	9.8	0.74	0.06	9.8	0.56	0.01	0.9	0.32
Ninole (3333)	1.38	10.6	0.99	0.06	9.9	0.89	0.01	1.0	0.22
Ninole (5000)	1.20	9.9	0.33	0.07	10.2	0.35	0.01	0.9	0.48
Ninole (6666)	1.00	9.1	1.02	0.06	9.4	0.66	0.01	0.9	0.08
Poamoho	1.45	10.8	0.17	0.07	10.1	0.06	0.01	0.9	0.14
Waiakea	0.54	7.7	0.32	0.04	8.4	0.28	0.01	0.8	0.01
N & P	1.35	10.5	0.19	0.07	10.2	0.29	0.01	1.0	0.04
N & W	0.76	8.4	0	0.05	9.1	0.14	0.01	0.9	0.03
N & P & W	0.97	9.3	0.02	0.05	9.3	0.10	0.01	0.9	0

While the density effects at Ninole were not significant, the CV_p for RGR decreased in a regular way from the low density to the high as competition increased (Table 6.3). The heritability estimates, however, did not follow the same trend, suggesting that the 5000 plants/ha density was somehow anomalous, as noted in Chapter 5 for various other traits.

As occurred with spininess, there were significant location effects on growth. When sites are combined interaction variances were generally large, occasionally larger than the progeny variances. In this analysis, Ninole and Poamoho presented similar levels of V_p but contrasting heritability estimates because of progeny by location interactions (Figure 6.1). Progenies B-1, B-2 and B-3 were nearly identical at Ninole but behaved

very differently at Poamoho. Progeny B-6 can be considered to be the most stable of these five progenies with respect to RGR.

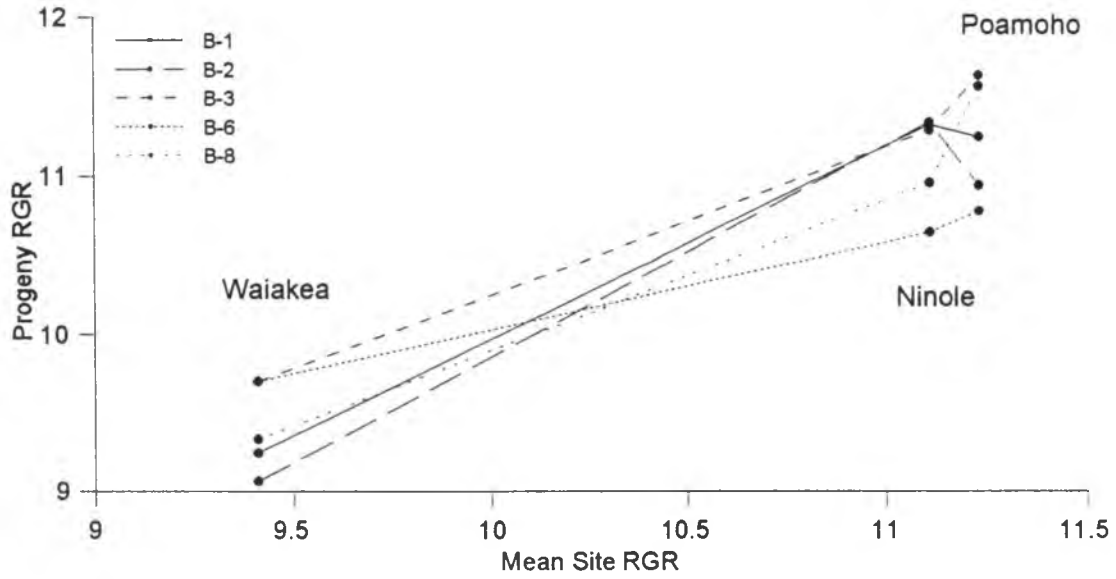

Figure 6.1. Variation in relative growth rates (RGR) of pejibaye over the experimental sites for those progenies present at all sites.

The Ninole heritability estimates for RGR and E_A are higher than those reported for oil palm (Chapter 2, Table 2.3) and lower for LAR. Part of the explanation may be the use of a uniform developmental phase for the analysis, since oil palm researchers normally use a uniform time interval for their work.

The growth period from nursery transplant to the first field evaluation at six months after planting was identified as important, because it was also highly correlated with precocity (Chapter 5, section 5.5). This uniform time period ended with the progenies in different developmental phases, however. The vigorous progenies had already entered the pinnate establishment phase, while the less vigorous progenies were still in the bifid eophyll establishment phase. The higher CV_p's are certainly due to the mixture of

developmental phases at the end of the time interval (Table 6.4). The heritability estimates are unusually large, strongly suggesting a high level of inbreeding in this germplasm. The large location effects were already evident after only six months in the field, principally because of periodic short droughts at Waiakea and labor problems at Poamoho that reduced the irrigation frequency. At Ninole, the 5000 plant/ha density again presented lower additive genetic variances than the other densities.

Table 6.4. The genetics of pejibaye growth from nursery to six months in the field. Phenotypic variances (V_P), coefficients of variation (CV_P), and narrow-sense heritabilities (h^2) (assuming no inbreeding (F = 0)) for relative growth rate (RGR), unit leaf rate (E_A) and leaf area ratio (LAR), at each site and over combinations of sites. Because inbreeding is likely (Chapter 7, section 7.3), $h^2_i = h^2$ (1-F), and F could = 0.50.

	RGR			E_A			LAR		
Site	V_P	CV_p	h^2	V_P	CV_p	h^2	V_P	CV_p	h^2
Ninole (all)	10.7	24.5	1.44	0.33	36.7	1.47	0.85	7.2	0.88
Ninole (3333)	10.9	24.6	1.78	0.33	37.2	1.87	0.91	7.5	1.00
Ninole (5000)	10.0	23.5	0.53	0.30	34.4	0.52	0.80	7.0	0.11
Ninole (6666)	8.96	23.0	1.71	0.28	35.4	1.70	0.66	6.4	1.00
Poamoho	7.01	22.3	0.30	0.17	29.5	0.34	0.61	6.2	0.20
Waiakea	4.91	25.3	0.86	0.12	39.7	0.75	0.91	7.2	0.96
N & P	8.36	23.5	0.25	0.23	33.3	0.27	0.71	6.7	0.22
N & W	6.99	25.0	0	0.20	37.9	0	0.74	7.0	0
N & P & W	6.59	22.5	0	0.18	32.8	0	0.67	6.4	0

The growth period from first to second field evaluation was used to provide a set of growth parameters for a uniform time interval. The growth parameters in this interval were not well correlated with early harvest (Chapter 5, section 5.6). The generally very high CV_p's are certainly due to the extreme variation in developmental stages at both the beginning and end of the time interval (Table 6.5). Location by progeny interactions

strongly depressed heritability estimates for all combinations of sites, as in the other analyses. At Ninole, the 5000 plant/ha density again presented lower additive genetic variances than the other densities.

Interestingly there was apparently more variability present for RGR and E_A than for LAR. In Chapter 5, two alternative growth strategies were identified: 1) high RGR was achieved via high E_A; 2) high RGR was achieved via high LAR. In the germplasm available here, there is more variability for E_A than for LAR, suggesting that genetic advance will be more easily obtained via selection for E_A. While oil palm breeders found significant variation in E_A, they also found considerable variation for LAR, as well as moderate heritabilities (Hardon et al. 1972).

Table 6.5. The genetics of pejibaye growth over a uniform time interval from the first to the second evaluations. Phenotypic variances (V_P), coefficients of variation (CV_P), and narrow-sense heritabilities (h^2) (assuming no inbreeding (F = 0)) for relative growth rate (RGR), unit leaf rate (E_A), and leaf area ratio LAR), at each site and over combinations of sites. Because inbreeding is likely (Chapter 7, section 7.3), $h^2_i = h^2 (1-F)$, and F could = 0.50.

	RGR			E_A			LAR		
Site	V_P	CV_p	h^2	V_P	CV_p	h^2	V_P	CV_p	h^2
Ninole (all)	31.9	55.0	0.78	0.54	52.7	0.64	1.41	15.8	0.69
Ninole (3333)	29.1	54.0	0.99	0.49	51.8	0.77	1.62	17.0	0.72
Ninole (5000)	38.4	61.3	0.41	0.63	57.7	0.49	1.24	14.9	0.12
Ninole (6666)	25.5	47.0	0.88	0.45	45.8	0.70	1.09	13.9	0.76
Poamoho	16.1	29.9	0.09	0.33	28.2	0	1.54	17.8	0.24
Waiakea	26.7	29.3	0	0.58	30.2	0.23	1.84	17.3	1.08
N & P	29.4	45.0	0.07	0.52	41.4	0.07	1.45	16.6	0.29
N & W	33.1	41.7	0.02	0.64	41.2	0.07	1.48	16.0	0.07
N & P & W	27.5	38.1	0.02	0.54	36.9	0.15	1.37	15.8	0

Although the germplasm evaluated here is a small sample of one population, there is additive genetic variability present that can be manipulated in a breeding program. The major problem is determining when growth should be evaluated for maximum selection gain. In the current case, the choice depends upon the correlation of growth rate with early harvest, and practicality. The highest correlation between earliness and RGR and E_A was from nursery transplant to harvest (Chapter 5, Table 5.8), but the correlation of RGR and E_A from nursery transplant to six months in the field was almost as high (Chapter 5, Table 5.4). Since the latter requires less time than the former, and the nursery environment is more easily controlled than the field, practicality suggests using the nursery to early field period.

The expected response from this selection can be estimated by $R = i * s * h^2$, where *i* is the intensity of selection, *s* is the phenotypic standard deviation of the trait in question, and h^2 is that trait's narrow-sense heritability (Falconer 1981, Brewbaker 1994). The proposal to use the nursery and early field period for evaluating growth for a pejibaye heart-of-palm improvement program, as opposed to the other two options discussed here, was tested in Table 6.6 for RGR and LAR at the 3333 plants/ha density at Ninole, assuming that 2% of the 189 plants are selected. The low density was chosen as an example because the 5000 plants/ha density was often anomalous. Two percent is used as a selection intensity because we also expect that tissue culturing of the selected plants will be a viable alternative within a few years.

The response to selection increases as *s* increases, suggesting that genetic variability is limited in this germplasm. As the time interval decreases and is less equatable with a

Table 6.6. Comparison of three growth interval's expected response (R) to selection for RGR and LAR at 3333 plants/ha at Ninole, assuming that 2% of the 189 plants are selected (i = 2.367), s is $V_P^{1/2}$ from Tables 6.3, 6.4, and 6.5, and h^2 is estimated with F = 0.5. The intervals are: n > h - nursery to harvest; n > 1 - nursery to first field evaluation; 1 > 2 - first to second field evaluation.

interval	current mean	s	h^2	R	R as %	future mean
RGR						
n > h	11.06	1.1735	0.49	1.36	12.3	12.42
n > 1	13.40	3.3009	0.89	6.95	51.9	20.35
1 > 2	10.00	5.3976	0.49	6.26	62.6	16.26
LAR						
n > h	10.73	0.1033	0.11	0.03	0.3	10.76
n > 1	12.74	0.9557	0.50	1.13	8.9	13.87
1 > 2	7.50	1.2738	0.36	1.09	14.5	8.59

developmental phase, *s* increases because more plants are in different developmental phases. If the magnitude of the response were the only criterion, essentially any early time interval would be sufficient for evaluating growth for selection purposes and gains would be expected to be rapid and large. As pointed out by Simmonds (1979) and Gupta (1992), however, very little response to selection for RGR and E_A has been realized. The reason may well be that the true *s* for these traits is that measured over the developmental phase, rather than that measured over a short time interval. For practical purposes, measuring growth in the nursery may be sufficient to identify plants for selection, but expectations of gain should be based on the variances of the target developmental phase(s) as a whole. Coleman et al.'s (1994) recommendation to evaluate phenotypes over both uniform time intervals and uniform developmental phases appears to be even more important for plant breeding than for ecology if breeders hope to improve a crop's

physiological efficiency. Practical issues, such as cost, may limit the widespread use of multiple interval evaluations, however, especially for minor crops.

6.4. Other Plant Traits

Early harvest (in days from nursery or field planting to first harvest) can be viewed as an easily measured form of RGR over the developmental phases of interest, i.e. from germination to first harvest. The heritability estimates for earliness (Table 6.7) were very similar to those for RGR itself (Table 6.3). Earliness, however, was less affected by density than RGR, as shown not only by the lack of significance of the density effect at Ninole, but also the lack of a trend of decreasing phenotypic variance with increasing density. As in previous analyses, the 5000 plant/ha density at Ninole presented anomalous results, and there are strong location effects that reduce the additive genetic variance over locations.

There was considerable variation in offshoot number and, at least at Ninole, an important percentage of the phenotypic variation appeared to be due to additive genetic effects (Table 6.7). There were significant location effects and location x progeny interactions (Figure 6.2), but, surprisingly this did not reduce the additive genetic variance estimated over locations as much as in previous analysis. Both Poamoho and Waiakea had more offshoots and apparently less additive genetic variance. The offshoot number heritabilities estimated at Ninole would allow for rapid gain in an improvement program, but most progenies do not need more offshoots. Rather they need changes in the way plant resources are partitioned to the offshoot, as pointed out in Chapter 5.

Table 6.7. The genetics of earliness (days to first harvest) in pejibaye and leaf and offshoot number at first harvest. Phenotypic variances (V_P), coefficients of variation (CV_P), and narrow-sense heritabilities (h^2) (assuming no inbreeding (F = 0)), at each site and over combinations of sites. Because inbreeding is likely (Chapter 7, section 7.3), $h^2_i = h^2 (1\text{-}F)$, and F could = 0.50.

	Precocity			Offshoot Number			Leaf Number		
Site	V_P	CV_g	h^2	V_P	CV_g	h^2	V_P	CV_g	h^2
Ninole (all)	1248	16.5	0.69	8.08	35.5	0.70	1.31	17.9	0.49
Ninole (3333)	1137	15.8	0.90	8.45	34.9	0.54	1.49	18.7	0.99
Ninole (5000)	1287	16.8	0.19	8.70	36.3	0.68	1.21	17.7	0.48
Ninole (6666)	1166	15.8	0.74	7.05	34.2	0.51	1.11	16.9	0.33
Poamoho	1106	17.0	0.08	5.61	27.8	0.06	1.43	17.8	0.14
Waiakea	802	11.4	0.50	8.36	30.2	0.06	0.81	15.7	0.01
N & P	1293	17.4	0.22	7.13	32.3	0.33	1.38	18.1	0
N & W	927	13.3	0.17	8.08	32.5	0.28	1.02	16.9	0
N & P & W	1006	14.5	0.07	6.71	29.6	0.21	1.19	17.5	0

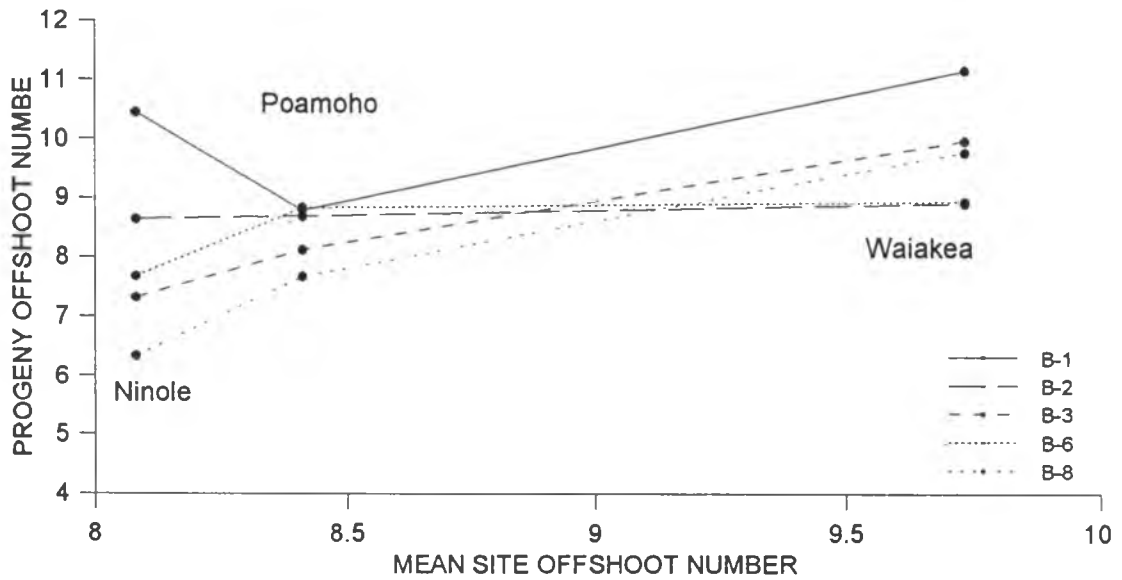

Figure 6.2. Variation in pejibaye offshoot number over the experimental sites for those progenies present at all sites.

Leaf number is an important trait effecting other growth parameters, either directly as in LAR, or indirectly as in RGR and E_A. As mentioned in Chapter 4, leaf number in these

trials is lower by two or three leaves than in similar concurrent trials in Brazil. The phenotypic variation in these Hawaiian trials is low (Table 6.7), as is additive genetic variance after correction for probable inbreeding. Strong block by progeny and location by progeny interactions reduced the additive genetic variance estimated at Poamoho, Waiakea and in the combined analyses. Given that environmental effects on leaf number are probably large, these results appear reasonable and genetic gains are unlikely to be rapid.

6.5. Heart-of-palm Yield Components

Yields of economic product generally have low heritabilities (Simmonds 1979, Gupta 1992). In pejibaye for heart-of-palm, there is a strong relationship between plant height and heart yield (Moreira Gomes & Arkcoll 1988), at least throughout the pinnate establishment and adult vegetative phases. Because of this relationship, and because plants were harvested at various heights due to the monthly harvest schedule, height was used as a covariate when analysing individual heart-of-palm weight. In all cases, height was a highly significant covariate. In the final combined analysis, the average harvest height was 1.44 m and all weights are ajusted to that mean. The regression of heart-of-palm weight on height was:

$$\text{weight (g)} = -5.5 + 128.1 \text{ height (m)}.$$

In Chapter 5 it was shown that heart length was the major component of heart-of-palm weight, either as a result of a more persistent sheath or reduced fibrousness in the

petiole. Height was a highly significant covariate for heart length also. The regression of heart length on height was:

length (cm) = 5.6 + 11.7 height (m).

There was considerable phenotypic variation for both heart-of-palm weight and length (Table 6.8). While there were also significant location effects and nearly significant location x progeny interactions (Figure 6.3), these did not dramatically reduce the additive genetic variances, as occurred in all previous analyses. Even the highest heritability estimate, when corrected for probable inbreeding, was extremely low. As in other crops, the response to selection for this yield trait would be extremely low. This, in fact, is the main reason for examining growth in such detail, as many of the growth-related traits have higher estimated heritabilities than heart weight and length.

Table 6.8. The genetics of pejibaye heart-of-palm weight and length at first harvest. Phenotypic variances (V_P), coefficients of variation (CV_P), and narrow-sense heritabilities (h^2) (assuming no inbreeding (F = 0)) were estimated at each site and over combinations of sites. Because inbreeding is likely (Chapter 7, section 7.3), $h^2_i = h^2$ (1-F), and F could = 0.50.

	Weight			Length		
Site	V_P	CV_p	h^2	V_P	CV_p	h^2
Ninole (all)	248	27.7	0.11	52.2	32.4	0.08
Ninole (3333)	244	27.8	0.06	52.7	33.3	0
Ninole (5000)	275	29.6	0	57.1	34.5	0
Ninole (6666)	249	26.9	0.22	46.4	29.7	0.10
Poamoho	315	27.9	0.08	55.3	27.9	0.08
Waiakea	237	30.5	0.04	57.9	34.4	0.03
N & P	310	29.3	0.22	56.6	32.7	0.17
N & W	279	31.6	0.07	59.2	35.2	0.11
N & P & W	293	30.2	0.11	57.1	33.6	0.10

The conditions at Waiakea severely reduced heart-of-palm weight (Figure 6.3), but not length (data not shown). Rather, heart-of-palm diameter was reduced at Waiakea, giving material that would be excellent for canning, rather than the fresh market. In terms of weight, progeny B-1 was relatively stable across sites; it was best at Waiakea and intermediate at both Ninole and Poamoho. Progeny B-3 apparently required a resource that was limiting at Waiakea, intermediate at Ninole and maximized at Poamoho; sunlight may be the resource, but this is still speculation at this point.

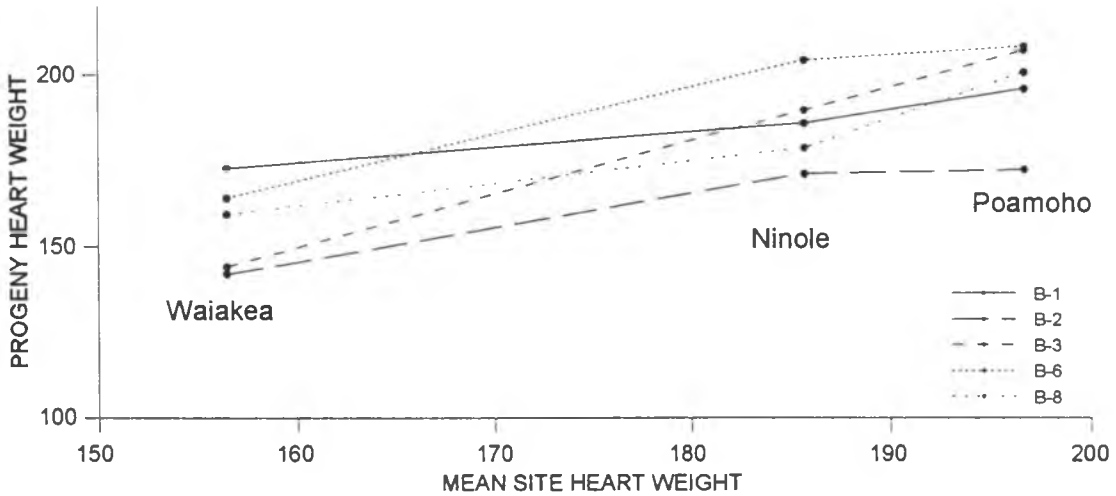

Figure 6.3. Variation in pejibaye heart-of-palm weight over the experimental sites for those progenies present at all sites.

6.6. Heart-of-Palm Quality Traits

The two quality traits that were evaluated were total soluble solids (TSS) and acridity (Table 6.9). Total soluble solids is an important positive sensory characteristic, especially if the heart or stem is to be used fresh. Acridity is the most important negative sensory trait (Cavalletto et al. 1994) and should be eliminated from improved materials because

of potential health problems, especially allergic reactions by a small percentage of consumers.

There was enormous variation in TSS, with a range from 3.6 to 13.0. There was more variation among harvests than among progenies, however. Use of harvest date as a covariate did account for significant amounts of variation, but progenies were seldom significantly different. Ninole at 5000 plants/ha was the only exception. Mean CV_p was 9.3 (data not shown). Consequently, estimates of additive genetic variance were extremely small and frequently negative, with heritabilities (F = 0) ranging from 0 to 0.15 (Ninole 5000) (data not shown).

Table 6.9. Pejibaye progeny mean total soluble solids (TSS) and acridity at each experimental site (Ninole is represented by the 5000 plants/ha density only).

	TSS			Acridity		
Progeny	Ninole	Poamoho	Waiakea	Ninole	Poamoho	Waiakea
B-0	8.25		9.64	0.0846		0.0355
B-1	8.24	8.93	9.47	0	0.1165	0.0500
B-2	8.46	8.99	9.76	0.0846	0	0
B-3	8.33	8.91	9.50	0	0.0628	0
B-5	7.98	8.80		0.0825	0.0500	
B-6	8.51	9.05	9.55	0.0628	0.0721	0.0957
B-7	8.55			0		
B-8	8.24	8.45	9.69	0	0	0.0275
B-9	8.31	9.09		0.0846	0	

There was slightly more variation for acridity (mean CV_p = 14.8), but progeny differences were never significant. Consequently, estimates of additive genetic variance

were extremely low or negative and heritabilities clustered around 0, with a maximum of 0.09 at Poamoho (data not shown).

The major limitation with the acridity data is that only one person (myself) evaluated acridity. I started the evaluations as relatively insensitive to acridity and became less sensitive as the number of palms tested mounted into the hundreds. MS C. Cavaletto (personal communication), Univ. Hawai'i at Manoa, determined that there is as much variation in people's sensitivity to acridity as there is in a plant population's acridity content. People can also lose their sensitivity with exposure, as I did.

Tomlinson (1990) affirms that all palms contain raphides, most of which are needle-like crystals of calcium oxalate. Dr. W. Sakai (personal communication), Univ. Hawai'i at Hilo, observed abundant raphide crystals in each of ten pejibaye samples examined, although half of them were acrid and half not, according to my sensory evaluation. Drs. W. Sakai, C.S. Tang, and R. Paull (personal communication), Univ. Hawai'i at Manoa, suspect that the acridic sensation is caused by a combination of the raphides and an activator enzyme. They are attempting to identify the enzyme to develop a bioassay for its presence and concentration. In the event that they are successful, a quantitative study of acridity should be done in the Hawaiian germplasm to identify plants with little or no acridity for cloning. A quantitative bioassay should also allow a better estimate of the additive genetic variance for this enzyme.

6.7. Conclusions

There were strong indications of inbreeding in the germplasm studied. Clement (1988) suggested that the domestication of pejibaye by Amerindians in Amazonia would have increased the inbreeding coefficient in most populations. Clement & Arkcoll (1984) found that two sub-samples of the Benjamin Constant population were highly self-fertile when self-pollinated. The history of the germplasm in the INPA agroforestry trial (Chapter 3, section 3.1) that supplied the current Hawaiian germplasm also suggests a high probability of inbreeding. The very high heritability estimates, frequently well in excess of 1.0, for traits that normally display moderate to low heritabilities, strongly support the existence of high levels of inbreeding.

Although the Benjamin Constant sample present in Hawai'i is small and many of the progenies may be closely related, there are useable levels of additive genetic variance for two of the most important traits: relative growth rate and earliness. These are very highly correlated traits, however, so only one of them is worth using. In terms of ease of measurement, earliness, measured as days to harvest, is the trait of choice.

Because earliness is due mostly to high RGR, however, the question of response is critical. Response depends strongly upon the variation present, which can be estimated in different ways, as well as the heritability. The lowest estimate, that over an entire developmental phase, may determine the response. Other, higher estimates, do not truly reflect variation for the trait itself; rather, they reflect variation among plants at different developmental stages. If this is true, response to selection will be slow, unless the outstanding plants are cloned.

All other important traits, i.e., spines on the petiole, offshoot number, leaf number, leaf area ratio, unit leaf rate, heart-of-palm weight and length, have much lower levels of additive genetic variance and will give a smaller response to selection. Careful selection of parents and locations for progeny testing may allow a reasonable response to selection, however, especially if combined with cloning.

The two quality traits have essentially no additive genetic variance. In the case of TSS, this lack of heritability is probably due to the fact that sugars are important energy molecules in most biosynthesis pathways and are not stored near the meristem. In the case of acridity, this lack of heritability may be an artifact of the insensitivity of the tester and should be reevaluated if a quantitative bioassay becomes available.

Chapter 7. Allozyme Variation

7.1. Introduction

The use of isoenzymes in plant improvement, and biology in general, has expanded rapidly in recent years (e.g., Tanksley & Orton 1983a, b, Soltis & Soltis 1989, Khanna 1991). Isoenzymes are efficient tools for examining genetic variation because they generally exhibit Mendelian inheritance, co-dominant expression, complete penetrance and an absense of pleiotropic and epistatic interactions (Weeden & Wendel 1989), all of which facilitate their genetic analysis. They may also serve as markers for quantitative traits, such as growth and yield (Stuber 1991, Koutou et al. 1992). In this chapter, electrophoretic methodology is refined for pejibaye, the amount and organization of the genetic variation present in the Benjamin Constant population sample is examined, and allozyme heterozygosity is correlated with plant morphology and growth parameters in the hopes of finding a relationship that can be useful in plant improvement.

In fruit tree improvement, isozymes have served several useful functions, including characterization and identification of cultivars, discrimination of selfed versus crossed progeny, determination of the somatic or zygotic origin of seedlings, documentation of the parentage of cultivars or interspecific hybrids, and examination of the genetic relationships among cultivars in germplasm collections (Torres 1989). In palms, isozyme analysis has mostly been used to assess the amount and distribution of genetic variability in geographic space (Ghesquière 1984, 1985, Ghesquière et al. 1987, Bennaceur et al. 1991), as well as in breeding populations (Ghesquière 1984, 1985).

Especially interesting in the context of this project is the correlation of allozyme heterozygosity with growth in trees. Mitton (1989) and Bush & Smouse (1992) review forestry research that strongly suggests that allozyme heterozygosity is often correlated with mean growth rate, especially at higher densities where competition is more intense. Aradhya & Phillips (1995) and others have not found useful correlations, however, raising the question of the universality of this relationship. Bush & Smouse (1992) point out that increases in population heterozygosity over time imply superior survival of heterozygotes and that for forest trees, a large component of ultimate survival is *the capacity for early growth* (citing Spurr & Barnes 1980) (emphasis added). It may be hypothesized from this that there is a relationship between allozyme heterozygosity and early growth which would be worth pursuing in pejibaye.

In this chapter, Rojas Vargas' (1993) work on refining electrophoretic methodology for pejibaye was expanded and adapted to starch gels. Most of the enzymes examined in other palms were tested. The amount and organization of allozyme diversity was examined through traditional measures, including the mean number of alleles present, the mean number of alleles per locus, the percentage of polymorphic loci, and the observed and expected heterozygosities in each progeny. Wright's fixation index and F-coefficients (Wright 1978) were used to verify the deviations from Hardy-Weinberg expectations, which might detect inbreeding in the formation of these progenies. Nei's (1975) genetic identity and distance were used to assess the similarity among the progenies and elaborate a dendogram to visualize the genetic relationships. Finally,

correlations between allozyme heterozygosity and important morphological and growth traits were examined to determine whether heterosis was important in trait expression.

7.2. Electrophoretic Methodology

Of the five gel- and tray-buffer systems tested, tris-citrate pH 7.5 and histidine-citrate pH 6.5 proved to be the most versatile, with tris-citrate somewhat better than histidine-citrate for the majority of the enzymes examined. For convenience, tris-citrate was used exclusively for electrophoresis.

Thirty-three enzyme systems were evaluated for activity, resolution and number of putative loci using both leaf and meristem extracts (Table 7.1). The enzymes adenylate kinase (ADK), catalase (CAT), formate dehydrogenase (FDH), hexokinase (HK), and mannose-6-phosphate isomerase (MPI) did not show any activity in either tissue and were not included in Table 7.1. Meristem extract always presented more activity, generally better resolution, and frequently more loci than leaf extract. The meristem was therefore chosen as the standard tissue for electrophoresis.

Ability to genetically interpret the banding patterns was the primary criterion for selecting enzymes for analysis. Some enzymes (e.g., IDH, MDH, ME, PGD, TPI) had good to excellent activity and resolution, but had so many bands that were often partially superimposed on each other that genetic interpretation was impossible. These enzymes could be used for phenotypic fingerprinting, but not for genetic analysis. Some enzymes were difficult to stain consistently (e.g., ACO, AMP, DIA, FBP, MNR) and were excluded. The following enzymes (with the putative loci that were interpretable) were

selected for use: AAT (1, 2, 3); ADH (1); G2DH (1); GDH (1); GPI (2, 3); LAP (1, 2); PGM (1, 2, 3); SKDH (1); UGPP (1, 2, 3). GPI 1, which should be a dimer, consistently appeared to be a monomer and was discarded from the analysis.

Table 7.1. Comparison of activity and resolution of enzymes extracted from meristem and leaf tissues of pejibaye for electrophoretic analysis.

		Meristem			Leaf		
Enzyme	Abrev.	Loci	Act.	Resol.	Loci	Act.	Resol.
Glucose-6-phosphate isomerase	GPI	3	9	9	3	7	7
Phosphoglucomutase	PGM	3	9	9	3	7	7
Shikimate dehydrogenase	SKDH	1	9	9	1	5	7
Malate dehydrogenase	MDH	4?	9	7	3?	7	7
Triose-phosphate isomerase	TPI	3?	7	7	3?	7	7
Malic enzyme	ME	4?	7	7	3?	5	7
Menadione reductase	MNR	2?	7	7	1	3	5
Alcohol dehydrogenase	ADH	2?	7	7	?	0	0
Uridine diphosphoglucose pyrophosphorylase	UGPP	3	7	5	3	7	5
Leucine aminopeptidase	LAP	2	7	5	2	5	3
Phosphoglutonate dehydrogenase	PGD	3?	7	5	2?	5	5
Aspartate aminotransferase	AAT	3	7	5	2	5	5
Acid phosphatase	ACP	6?	5	7	3?	1	1
Isocitrate dehydrogenase	IDH	3?	5	7	?	0	0
Diaphorase	DIA	2?	5	5	1	1	3
Glutamate dehydrogenase	GDH	1	5	5	1	3	5
Glycerate-2-dehydrogenase	G2DH	1	5	5	1	5	5
Glucose-6-phosphate dehydrogenase	G6PDH	1?	5	3	1?	3	3
Glyceraldehyde-3-phosphate dehydrogenase	G3PDH	1?	5	3	?	0	0
Fructose-bisphosphatase	FBP	1	5	3	?	0	0
Arginine aminopeptidase	AMP	1	5	3	?	0	0
Peroxidase	PRX	1	5	1	1	3	1
Esterase	EST	2?	3	5	1?	1	3
Superoxide dismutase	SOD	1	3	5	?	1	1
Alkaline phosphatase	ALP	1?	3	3	?	0	0
Sorbitol dehydrogenase	SDH	1	3	3	?	0	0
Aconitate hydratase	ACO	2?	3	3	?	0	0
Endopeptidase	ENP	1?	1	1	?	0	0

Both activity and resolution were scored on an ordinal scale of 0, 1-9, with 0 - no activity or resolution, 1 - very slight activity but not enough to use, 3 - barely enough activity to use, 5 - enough activity, 7 - good activity, 9 - excellent activity.

7.3. Genetic Variation

Six of the 17 putative isoenzyme loci were monomorphic. The remaining 11 loci contained a total of 29 alleles, yielding an average of 2.06 alleles/locus for the Benjamin Constant population as a whole. This moderate level of variation in zymograms (banding patterns) is illustrated in Figure 7.1. All of the polymorphic enzymes were monomeric. Three different zymograms were observed for AAT2 & 3, GDH, LAP 1 & 2, and PGM3. Four zymograms were observed for AAT1 and G2DH, while PGM1 and UGPP3 had six, and SKDH had seven zymograms.

Eight of the 35 alleles were rare (Table 7.2), occurring in only one or a few progenies with less than 5% frequency on average. Consequently, the eight Benjamin Constant progenies averaged only 1.65 alleles per locus (Table 7.3). The percentage of polymorphic loci ranged from 12% in progeny B-3 to 47% in progeny B-8, considering a locus to be polymorphic if the frequency of the most common allele did not exceed 0.95. When a higher threshold frequency of 0.99 was used to define the monomorphic state, a larger percentage of loci were identified as polymorphic (53-59%).

In an analysis of isozyme variation in *Phoenix dactylifera*, Bennaceur et al. (1991) reported a mean of 2.03 alleles per locus in four populations, 2.28 alleles per locus over the four, and 71-100% polymorphic loci per population. In *Elaeis guineensis*, Ghesquière (1985) reported a mean of 2.33 alleles per locus for five African populations and 3.47 alleles per locus overall, with 73-100% polymorphic loci per population. In *Elaeis oleifera*, Ghesquière et al. (1987) reported a mean of 1.55 alleles per locus for seven

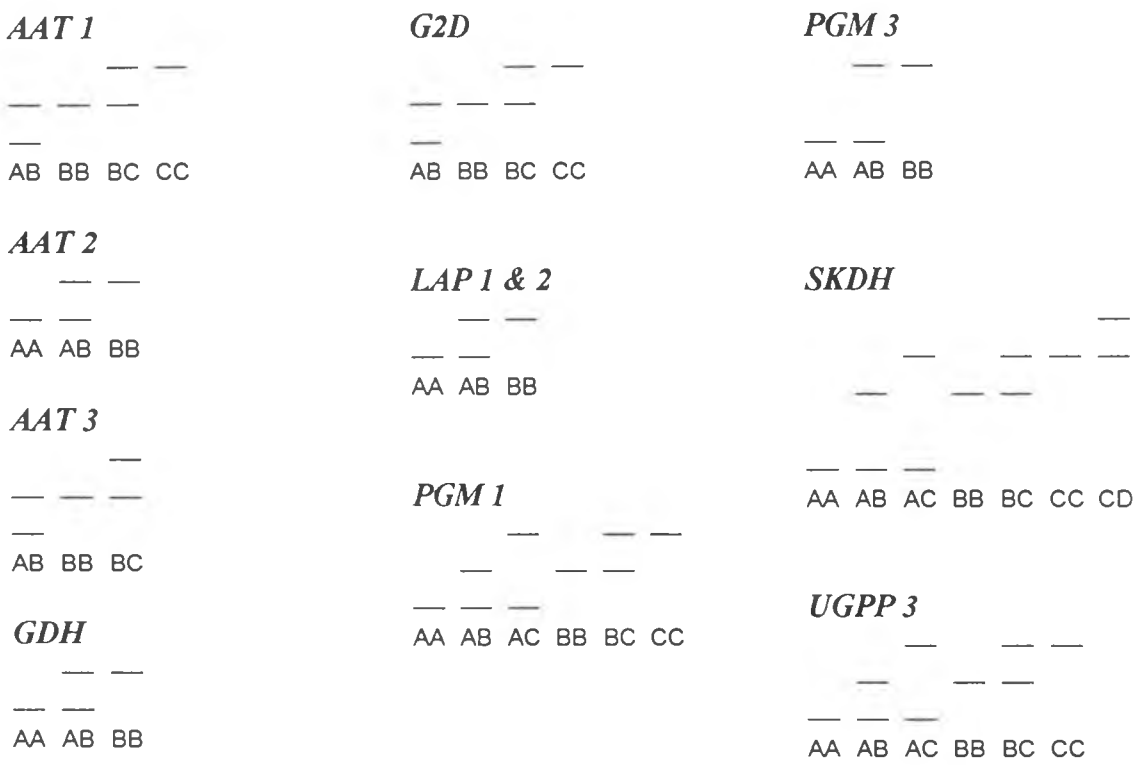

Figure 7.1. Schematic representation of the zymograms observed at 11 putative loci of seven enzyme systems in pejibaye. ADH 1, GPI 2 & 3, PGM 2, and UGPP 1 & 2 were monomorphic. The cathodic (-) pole and origin of the pejibaye tissue extracts are at the bottom.

Amazonian regions of diversity, 2.21 alleles per locus overall, and 21-79% polymorphic loci per population.

Observed heterozygosity in the Benjamin Constant progenies ranged from 0.038 to 0.099, with a mean of 0.074 (Table 7.3). The observed heterozygosity was much lower than that for *P. dactylifera* (0.411-0.50) (Bennaceur et al. 1991), *E. guineensis* (0.228-0.465) (Ghesquière 1985), or *E. oleifera* (0.21-0.36) (Ghesquière et al. 1987). The observed range in this population is even lower than the within-population heterozygosity of *Zea mays* landraces in Mexico (0.18) (Doebley et al. 1985), a fully domesticated out-

Table 7.2. Frequency of isozyme alleles at each putative locus for each pejibaye progeny. Number of samples analysed is in parentheses below the progeny identification.

Enzyme		B-0	B-1	B-2	B-3	B-5	B-6	B-8	B-9
Locus	Allele	(34)	(31)	(30)	(31)	(28)	(26)	(29)	(33)
AAT1	A	0.015	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	0.986	1.0	1.0	1.0	0.982	1.0	0.983	1.0
	C	0.0	0.0	0.0	0.0	0.018	0.0	0.017	0.0
AAT2	A	0.074	0.323	0.117	0.145	0.321	0.192	0.069	0.485
	B	0.926	0.677	0.883	0.855	0.679	0.808	0.931	0.515
AAT3	A	0.015	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	0.970	0.968	0.967	0.952	0.982	0.981	0.983	0.970
	C	0.015	0.032	0.033	0.048	0.018	0.019	0.017	0.030
G2D1	A	0.0	0.0	0.017	0.0	0.0	0.019	0.0	0.0
	B	1.0	1.0	0.983	0.968	0.982	0.981	1.0	1.0
	C	0.0	0.0	0.0	0.032	0.018	0.0	0.0	0.0
GDH1	A	0.882	0.968	0.983	0.984	0.768	0.769	0.897	0.848
	B	0.118	0.032	0.017	0.016	0.232	0.231	0.103	0.152
LAP1	A	0.0	0.032	0.017	0.032	0.0	0.019	0.052	0.015
	B	1.0	0.968	0.983	0.968	1.0	0.981	0.948	0.985
LAP2	A	0.235	0.081	0.267	0.097	0.143	0.135	0.103	0.212
	B	0.765	0.919	0.733	0.903	0.857	0.865	0.897	0.788
PGM1	A	0.059	0.242	0.033	0.016	0.304	0.135	0.207	0.045
	B	0.951	0.758	0.967	0.984	0.696	0.865	0.793	0.939
	C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.015
PGM3	A	0.676	0.887	0.983	0.952	0.964	0.942	0.914	0.955
	B	0.324	0.113	0.017	0.048	0.036	0.058	0.086	0.045
SKDH1	A	0.015	0.016	0.017	0.0	0.054	0.0	0.052	0.045
	B	0.029	0.016	0.0	0.0	0.018	0.0	0.0	0.015
	C	0.956	0.968	0.983	1.0	0.929	0.981	0.948	0.939
	D	0.0	0.0	0.0	0.0	0.0	0.019	0.0	0.0
UGPP3	A	0.015	0.048	0.033	0.0	0.036	0.0	0.069	0.061
	B	0.971	0.935	0.950	0.984	0.893	0.981	0.828	0.864
	C	0.015	0.016	0.017	0.016	0.071	0.019	0.103	0.076

breeder, and is on par with the total heterozygosity observed in *Capsicum annum* (0.074-0.105) (McLeod et al. 1983), a fully domesticated inbreeder. In summary, this Benjamin Constant sample shows a striking lack of heterozygosity, to a degree more characteristic of inbred crops.

Table 7.3. Allozyme polymorphism at 17 putative loci in 9 enzyme systems in the eight Benjamin Constant progenies of pejibaye in Hawaii.

	Number of	Mean±SE Alleles /	% Loci Polymorphic[a]		Mean±SE Heterozygosity	
Progeny	Alleles	Locus	0.95	0.99	Observed	H-W Exp.
B-0	29	1.71±0.19	29.4	52.9	0.078±0.025	0.088±0.033
B-1	28	1.65±0.17	29.4	52.9	0.085±0.034	0.091±0.033
B-2	28	1.65±0.15	17.6	58.8	0.041±0.013	0.059±0.025
B-3	26	1.53±0.12	11.8	52.9	0.038±0.012	0.049±0.017
B-5	29	1.71±0.17	35.3	58.8	0.099±0.031	0.118±0.039
B-6	27	1.59±0.12	29.4	58.8	0.072±0.028	0.086±0.030
B-8	28	1.65±0.15	47.1	58.8	0.091±0.031	0.093±0.027
B-9	29	1.71±0.19	35.3	52.9	0.087±0.030	0.104±0.036
overall	35	2.06			0.074	0.086

[a] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95 or 0.99, respectively.

A high level of sib-mating in this experimental population over the last three generations has certainly contributed to the loss of heterozygotes. However, it is unlikely, by itself, to explain the full magnitude of the depletion in genetic variability. I feel that it is safe to assume that the original Benjamin Constant population itself is also inbred, based on the practices used by Amazonian peoples to domesticate plants. These practices include strong selection pressure, small population size, short generations (dictated by swidden abandonment), and a high likelihood of sibs planted near each other (Clement

1988). Therefore, the low observed heterozygosity is at least partially the result of domestication *in situ* and reenforces Mora Urpí & Clement's (1988) suggestion that the Benjamin Constant population is highly derived. This also supports Clement's (1992) proposal that pejibaye is a full domesticate, since a reduction in allozyme heterozygosity accompanies domestication in all crops examined to date (Doebley 1989).

Wright's fixation index estimates the excess of heterozygotes or homozygotes at a given locus relative to values predicted by the Hardy-Weinberg principle (Table 7.4) (Hedrick 1985, Swofford & Selander 1989); its mean value was obtained from the observed and expected heterozygosities in Table 7.3. The index ranges from -1, indicating an excess of heterozygotes, to 1, indicating an excess of homozygotes, relative to Hardy-Weinberg expectations. At the heterozygous loci in the eight progenies examined, several loci, e.g., AAT2, GDH, LAP1 & 2, and PGM3, presented occasional significant excesses of homozygotes. All eight progenies had excesses of homozygotes, suggesting an inbred conditon, although only progeny B-2 presented a significant mean excess of homozygotes.

Wright's F-coefficients provide a description of the distribution of genetic variation within the total population (Hedrick 1985). The mean values for F_{IS} and F_{IT} were 0.125 and 0.184, respectively. F_{IS} measures the deviations from Hardy-Weinberg equilibrium within each progeny, and F_{IT} measures the same deviations within the population of progenies. Both measures suggest an excess of homozygotes, which confirms, at least, the sib-mating among the parents of these progenies. The coefficient F_{ST}, which measures the genetic differentiation among the progenies, presented a low value of 0.068,

indicating that only a small amount of interprogeny differentiation exists in this population (Hedrick 1985). This is in agreement with the history of selection and probable inbreeding.

Table 7.4. Wright's fixation index at each heterozygous locus and overall. The empty cells in the table indicate fixation.

enzyme	B-0	B-1	B-2	B-3	B-5	B-6	B-8	B-9
AAT1	-0.015				-0.018		-0.018	
AAT2	-0.079	-0.033	0.515*	0.350	0.345	0.257	0.463	0.151
AAT3	-0.133	-0.033	-0.034	-0.051	-0.018	-0.020	-0.018	-0.031
G2D1			-0.017	-0.033	-0.018	-0.020		
GDH1	-0.133	-0.033	-0.017	-0.016	0.499*	-0.083	-0.115	0.293
LAP1		1.0*	-0.017	-0.033		-0.020	-0.055	-0.015
LAP2	0.183	-0.088	0.489*	0.262	-0.167	0.505*	-0.115	0.275
PGM1	-0.062	-0.143	-0.034	-0.016	0.071	-0.156	-0.261	-0.052
PGM3	0.328	0.517*	-0.017	0.650*	-0.037	0.646	0.781**	0.651*
SKDH1	-0.036	-0.025	-0.017		-0.062	-0.020	-0.055	-0.052
UGPP3	-0.023	-0.055	-0.040	-0.016	-0.091	-0.020	-0.151	-0.114
mean[a]	0.113	0.066	0.305¤	0.224	0.161	0.163	0.022	0.163

* χ^2 for difference between observed and expected was significant at $p < 0.05$; ** χ^2 significant at $p < 0.01$

[a] The mean is calculated from the observed and H-W expected heterozygosities over all loci (Table 7.3) as: F = (exp-obs)/exp. The significance of F is determined with a t-test. ¤ is significant at $p < 0.05$

With the small amount of genetic differentiation present in these eight progenies, it was not surprising to find that Nei's genetic distances and identities (Table 7.5) showed that the progenies are very closely related. In fact, they were almost identical at the 17 loci examined (Figure 7.2).

Table 7.5. Nei's (1978) unbiased genetic distances (below diagonal) and unbiased genetic identities (above diagonal) for the eight Benjamin Constant pejibaye progenies.

	B-0	B-1	B-2	B-3	B-5	B-6	B-8	B-9
B-0	*****	0.990	0.994	0.994	0.987	0.994	0.994	0.985
B-1	0.010	*****	0.993	0.996	0.998	0.997	0.996	0.995
B-2	0.006	0.007	*****	0.999	0.990	0.996	0.996	0.991
B-3	0.006	0.004	0.001	*****	0.991	0.997	0.997	0.991
B-5	0.013	0.002	0.010	0.009	*****	0.999	0.996	0.995
B-6	0.006	0.003	0.004	0.003	0.001	*****	0.998	0.994
B-8	0.006	0.004	0.004	0.003	0.004	0.002	*****	0.988
B-9	0.015	0.005	0.009	0.009	0.005	0.006	0.012	*****

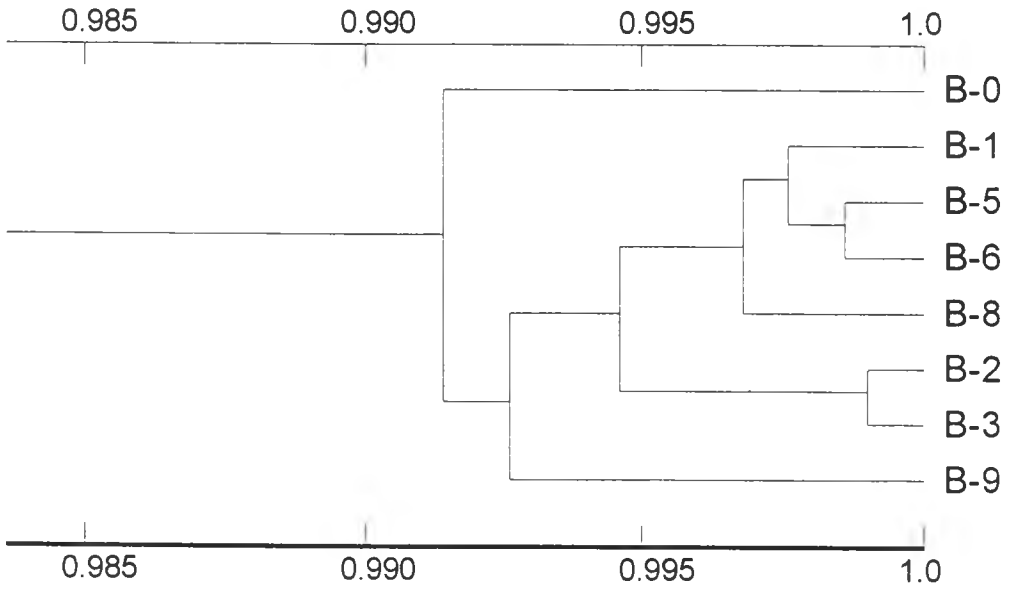

Figure 7.2. Dendrogram generated using Nei's unbiased genetic identities between the eight Benjamin Constant pejibaye progenies. The cophenetic correlation coefficient = 0.690.

7.4. Correlations with Growth Parameters and Morphological Traits

Among these Benjamin Constant progenies, mean allozyme heterozygosity was not correlated with the important morphological traits and growth parameters examined in Chapters 5 and 6 (Table 7.6). While there were two significant correlations with allozyme heterozygosity, namely, in progeny B-5 with total edible weight ($r = 0.465$) and in B-8 with spines on the petiole ($r = -0.75$), overall there is a lack of correlation. The two significant correlations are probably artifacts of small sample size. This is especially likely for the spine correlation, since a positive correlation would have been expected according to current thinking about the genetics of spines, i.e., spines are conditioned by dominant factors and spinelessness is the homozygous recessive condition.

Since most of the phenotypic variation of the traits listed in Table 7.6 has a large environmental component (see Chapter 6), and the very low heterozygosities strongly suggest a recent history of inbreeding, the absense of correlations that would suggest heterosis is not surprising. This small pejibaye sample is composed of closely related, highly inbred germplasm, in what is considered an allogamous species. Thus, the lack of heterotic affects is an additional confirmation of the high degree of inbreeding in the recent history of this germplasm.

7.5. Conclusions

Starch gel electrophoresis is an efficient way of examining genetic variation in pejibaye. The best gel-tray buffer combination was tris-citrate, pH 7.5, although histidine-citrate, pH 6.5, was nearly as good. The palm meristem was the best tissue for

obtaining abundant enzyme extracts. Twenty-eight of the 33 enzyme systems examined gave some to good activity and resolution. Nine systems, with 17 putative loci, were chosen for good activity, resolution and ease of genetic interpretation.

Table 7.6. Progeny sample mean heterozygosities and correlations between individual mean heterozygosity and growth parameters and physical traits at Ninole.[a] Two mean correlations are presented: using all individuals as though there was only one population; using progeny means for each trait.

	Progenies								all	prog.
	B-0	B-1	B-2	B-3	B-5	B-6	B-8	B-9	indiv.	mean
hetero	0.080	0.112	0.052	0.032	0.091	0.077	0.091	0.078	0.077	0.077
SD	0.066	0.076	0.051	0.053	0.060	0.058	0.063	0.046	0.063	0.023
RGR	0.006	0.242	-0.277	-0.398	0.073	0.528	-0.024	0.026	-0.047	-0.166
NAR	-0.165	0.331	-0.292	-0.357	0.098	0.550	0.078	-0.101	-0.046	-0.187
LAR	0.216	-0.399	0.100	0.071	-0.053	-0.256	-0.228	0.289	-0.007	-0.059
precocity	-0.060	-0.256	0.265	0.399	-0.090	-0.486	-0.019	-0.069	0.019	0.116
spines		0.153	0.381	-0.149	0.254	-0.032	-0.750	0.073	-0.024	-0.204
offshoots	-0.226	0.100	0.022	-0.398	-0.124	-0.183	0.201	0.078	0.042	0.514
heart wt	-0.259	0.037	0.337	0.179	0.085	-0.118	-0.194	-0.075	0.010	0.222
total wt	0.135	0.043	0.288	-0.144	0.465	0.190	-0.083	-0.166	0.104	-0.131
critic r	0.398	0.456	0.398	0.514	0.414	0.553	0.482	0.368	≈0.2	0.707
n	25	19	25	15	23	13	17	29	166	8

[a] Poamoho and Waiakea were omitted to avoid location effects on the traits and parameters.

Six putative loci were found to be monomorphic in the eight Benjamin Constant progenies examined, while the remaining 11 putative loci, were polymorphic. Thirty-five alleles were found over the 17 loci. There was an average of 1.65 alleles per locus in each progeny and 2.06 over the eight progenies. The percentage of loci that were polymorphic ranged from 12 to 47, with the criterion that a locus was considered to be polymorphic if

the frequency of the most common allele did not exceed 0.95. Observed heterozygosity ranged from 0.038 to 0.099, with a mean of 0.074, and was in good agreement with Hardy-Weinberg expectations. Wright's fixation index showed that several loci presented occasional excesses of homozygotes in specific progenies and that one progeny had a significant excess of homozygotes. Wright's $F_{ST} = 0.068$, highlighted the lack of differentiation among progenies in this sample. Nei's genetic identities for the eight progenies were close to unity, ranging from 0.985 to 0.999. In sum, this sample of the Benjamin Constant population shows a striking lack of heterozygosity, so much so that it is more comparable to a typical inbred crop than a typical outbred crop. Given the probable inbreeding in the recent history of this germplasm, the correlations between allozyme heterozygosity and morphological traits and growth parameters were generally not significant and did not show any trend that might suggest heterosis.

Chapter 8. Summary

8.1. Introduction

This analysis of the morphology, growth and yield of pejibaye in Hawai'i is the first of its kind. As such, it has raised as many questions as it has answered. Nonetheless, with the information obtained, it is possible to plan a production and improvement program for pejibaye in Hawai'i, in the event that the fledgling heart-of-palm industry expands sufficiently in the coming years to warrant the investment. In this chapter, I summarize the major conclusions and questions identified in the study and show how they can be used to guide agronomic research on the crop and to develop a crop ideotype to guide an improvement program.

8.2. Morphology

The morphological descriptions of the seedling, establishment and early adult-vegetative phase plants provide baseline information about pejibaye in Hawai'i and are also useful elsewhere. Leaf number was found to be much lower than expected at the end of the establishment phase (mean of 6-7 leaves in most progenies; 7-8 in progeny B-9), especially in terms of currently accepted notions of palm growth. A comparison with leaf number in a similar plantation in Brazil found a deficiency of 2-4 leaves in Hawai'i; the Brazilian plantation had a mean of 10 leaves and received much less fertilizer. Because leaf number in Hawai'i was higher during the early establishment phase, when the palms were receiving micronutrients with the macronutrients, than at the end of the establishment phase, when the palms were receiving only macronutrients, the possibility

of micronutrient deficiency must be examined. An increase in leaf number should result in an increase in growth rates and precocity and would definitely be cost effective. This hypothesis is being tested at Ninole, where micronutrients are being applied with the macronutrients.

Offshoot number declined dramatically from the first (mean of 6.5, range of 0 to 18) to the second harvest (mean of 2, range of 0 to 10). Offshoot management was probably the major cause of this decline, since the full compliment of offshoots was allowed to develop up to first harvest so that each plant's full potential could be evaluated. The often large number of offshoots resulted in severe competition among them, so that when they were finally pruned to keep two in each clump, they only had three to four leaves each. This severe competition may have caused a deficiency in energy partitioning to each offshoot's own buds, resulting in significant bud abortion. The validity of this hypothesis can easily be verified by observing offshoot production in a commercially managed plantation, where among-offshoot competition is not allowed to become as excessive as at first harvest in this project.

A second factor that may be influencing offshoot numbers is that, once liberated from the main stem, offshoots grow faster than the seed-derived main stem, thus allowing less time for new offshoot differentiation. One year after field planting, but four to twelve months before first harvest, the palms averaged 4.8 offshoots. At second harvest, approximately one year after first harvest, the palms averaged 2 offshoots. The faster growth of the second cycle stems may change the way resources are partitioned to offshoot buds, resulting in fewer offshoots for the third cycle. This hypothesis would

predict that offshoot numbers in the third and fourth harvest cycles would remain below first-cycle means but should be higher than second-cycle means (because of the interaction with management).

8.3. Allometry

Previously developed prediction equations for bifid eophyll leaf area and pinnate leaf area and biomass were validated, but slight modifications were found for each. These modifications may be population or environment specific. Consequently, each equation should be validated before use on other populations in tropical America. A prediction equation for bifid eophyll biomass was developed. The recommended prediction equations are:

Bifid eophyll:

$$L_A = -36.2 + 1.0L_{RA} + 5.0R_{W\cdot T}$$

$$L_W = 0.0129\, L_{RA}^{0.83} \cdot R_{W\cdot T}^{0.333}$$

Pinnate leaf:

$$L_A = -0.113 + 0.304\, L_{RA} + 0.421\, R_L$$

$$L_W = -193 + 22.9\, R_T$$

Plant height was the best predictor of both whole plant leaf area and biomass in both sub-phases. Leaf number was often a useful auxiliary predictor. The prediction equations that were used to study growth in Hawai'i are:

Bifid eophyll sub-phase:

$$L_A = 0.157 \cdot H^{1.61} \cdot L^{1.85}$$

$$W = 0.027 \cdot H^{1.93} \cdot L^{2.05}$$

Pinnate sub-phase:

$$L_A = 2.94 \cdot H^{1.36} \cdot L^{0.313}$$

$$W = 1.22 \cdot H^{1.77}$$

All four whole plant prediction equations must be used with caution. Not only was the sample size used to generate them relatively small, especially for the bifid eophyll sub-phase predictors, but the plants used to generate the equations had fewer leaves than expected. Consequently, these equations must be validated before use in other projects.

The proposal of two sub-phases within the establishment phase defined by Tomlinson (1990) was shown to be both biologically and statistically valid. Biologically, leaf form changes from bifid eophyll to pinnate as the plant passes through the height interval of 0.2 to 0.3 m, rarely to 0.4 m. Statistically, the slopes of the regressions that relate height to whole plant biomass were significantly different for the two sub-phases. Although the slopes of the regressions that relate height to leaf area suggest the same difference, they were not statistically different.

8.4. Growth and Yield

Seed traits and germination parameters presented unexpected correlations among themselves that suggested that seed management before sowing was not uniform. This lack of adequate management, in turn, made other conclusions based on germination of dubious value. Nonetheless, the correlation between the length of the largest bifid eophyll

at transplant and leaf area ratio over the period from transplant to harvest suggests that some selection might be usefully practiced early in the nursery period.

There were no density effects, nor border row effects within the plot at Ninole. This suggests that either plot size was too small (quite probable), or that there were too few leaves and LAI was not high enough to create a highly competitive environment, or that these densities did not reduce vegetative growth of the plants. In *Elaeis guineensis*, Corley (1973) found that vegetative growth was little effected by increasing plant density up to very high densities (high LAI's). Fruit yield dropped off rapidly, however, after the optimum density. This suggested to Corley that *E. guineensis* partitions more to vegetative growth when stress increases, such as with increased density and interplant competition. At the very high densities used for heart-of-palm production, leaf number was expected to show density effects, but most progenies did not present decreased leaf number. The lack of density and border row effects allowed the use of all plants for these growth studies and the subsequent genetic analysis.

Growth in the nursery and the first six months in the field was highly correlated with earliness, measured as days to harvest. This suggests that selection can be practiced at the time of field planting. In this case, plant height and leaf number can be used to identify those plants and progenies with greatest potential for vigorous growth and precocious harvest.

There was considerable variation in growth and growth parameters during all of the intervals examined, both over uniform time periods and over uniform developmental phases. Uniform time periods presented greater variation among the progenies than

uniform developmental phases. This makes biological sense, since, during any given time interval, different progenies (and even different plants within a progeny) are at different developmental phases, which implies different sizes and rates of growth.

Relative growth rate (RGR) estimated over the period between the nursery and first harvest was found to be highly and negatively correlated with earliness, as expected. Less expected was the fact that unit leaf rate (E_A) explained the majority of this relationship. Unfortunately, E_A normally has low heritability because of the numerous environmental factors that influence it (Gupta 1992). Additionally, the true magnitude of RGR and E_A was never determined because of the lower than expected leaf number. Consequently, these parameters should be reevaluated in an environment with optimum levels of all nutrients. This can best be done in the nursery and measured over the bifid eophyll establishment sub-phase.

There was some variation in the way progenies partitioned photoassimilates. Among the three most precocious progenies up to first harvest at Ninole, two progenies (B-0, B-1) had higher E_A, while the third progeny (B-9) had only an average E_A but partitioned more to leaf area, resulting in a higher LAR. These two ways of partitioning resources are commonly observed in crops and non-crop plants (Causton & Venus 1981). Over the entire experimental period at Ninole, progeny B-9 maintained its rapid growth, which suggests that LAR may be the most important growth parameter in that environment. At Poamoho and Waiakea, however, progeny B-9 presented only average growth, which suggests that high LAR may not be most important in all environments.

A third way of partitioning resources was observed for the least precocious progeny, B-5. In the two better environments, Ninole and Poamoho, this progeny consistently performed the worst, except in individual plant heart-of-palm weight. At Waiakea, however, which was the most stressful environment in this project, progeny B-5 performed amongst the best. At Ninole, B-5 was observed to be relatively stable during periods of stress, so that its better performance at Waiakea may be due to this stability of growth. It doesn't grow well, but when all other progenies are growing poorly because of environmental stress, B-5 appears to be better.

Potential heart-of-palm yields at Ninole were acceptable, at about 900 kg/ha for the first harvest. Actual yields, due to pig damage and the occasional extremely slow growing plant, were somewhat less. Nonetheless, these yields show that pejibaye is well adapted to the Hamakua Coast of the Island of Hawai'i. Regrowth after first harvest was strong and, with two stems per clump, 18 month yields were about 1.4 t/ha. When edible stem and edible leaf are included, actual yields were about 3 t/ha of marketable product.

In general, the use of classical growth analysis methods worked very well. They allowed the identification of different growth strategies, as well as considerable among progeny phenotypic variation, both of which may be amenable to selection in an improvement program. The use of these methods is strongly recommended for future research in pejibaye, both in Hawai'i and in tropical America.

8.5. Quantitative Genetic Analysis

There were strong indications of high levels of inbreeding in the germplasm studied. The heritabilities estimated with the assumption of F = 0 were frequently well in excess of 1.0 for traits that normally display moderate to low heritabilities. These high estimates strongly supported the hypothesis that selection during domestication and during the recent selections for spinelessness has resulted in high levels of inbreeding in these progenies. This hypothesis was confirmed by analysis of allozyme variation (see below).

Although the Benjamin Constant sample present in Hawai'i is small and many of the progenies are closely related, there are useable levels of additive genetic variance for two of the most important traits: RGR and earliness. These are very highly correlated traits, however, so only one of them is worth using. In terms of ease of use, earliness, measured as days to harvest, is the trait of choice.

Because earliness (days from field planting to harvest) is due principally to high RGR, the question of how to accurately measure RGR is critical. Response to selection for high RGR depends upon the amount of variation present, the heritability of the trait, and the selection intensity. The variation present can be estimated in different ways. Because harvest occurs at the end of a developmental phase, measurement of RGR over the entire developmental phase (from seedling to harvest, at the beginning of the adult-vegetative phase) gives the least inflated and most accurate estimate of variation. If this is true, response to selection will be slow, since, by this measure, variation of RGR is low. Cloning of outstanding plants would overcome this limitation.

All other important morphological traits, i.e., spines on the petiole, offshoot number, leaf number, leaf area ratio, unit leaf rate, and heart-of-palm weight and length have much lower levels of additive genetic variance, probably because of the high level of inbreeding in the history of this population. Consequently, these traits will give a smaller response to selection. Careful selection of parents, and locations for progeny testing, may allow a reasonable response to selection. Again, cloning can overcome this limitation, and further germplasm introductions are necessary.

The two quality traits had essentially no additive genetic variance. In the case of total soluble solids, this lack of heritability is probably due to the fact that sugars are important energy molecules in most biosynthesis pathways and their quantity is in constant flux in the meristem. Their local abundance in vegetative tissues that are not specialized for storage certainly depends upon the environmental conditions for plant growth at any given time.

In the case of acridity, this lack of heritability may be an artifact of the insensitivity of the tester and should be reevaluated if a quantitative bioassay becomes available. The identification of totally non-acrid plants might be very important in Hawai'i, where many chefs would like to use a completely fresh product.

8.6. Allozyme Variation

Starch gel electrophoresis is an efficient way of examining isoenzymatic variation in pejibaye. The best gel-tray buffer combination was tris-citrate, pH 7.5, although histidine-citrate, pH 6.5, was nearly as good. The palm meristem was the best tissue for

obtaining abundant enzyme extracts. Twenty-eight of the 33 enzyme systems examined gave some to good activity and resolution. Nine systems, with 17 putative loci, were chosen for good activity, resolution and ease of genetic interpretation.

Six putative loci were found to be monomorphic in the eight Benjamin Constant progenies examined, while the remaining 11 putative loci were polymorphic. Thirty-five alleles were found over the 17 loci. There was an average of 1.65 alleles per locus in each progeny and 2.06 over the eight progenies. The percentage of polymorphic loci ranged from 12 to 47, with the criterion that a locus was considered to be polymorphic if the frequency of the most common allele did not exceed 0.95.

Observed heterozygosity ranged from 0.038 to 0.099, with a mean of 0.074, and was in good agreement with Hardy-Weinberg expectations. Wright's fixation index showed that several loci presented occasional excesses of homozygotes in specific progenies. Wright's $F_{ST} = 0.068$, highlighted the lack of genetic differentiation among progenies in this sample. Nei's genetic identities were close to unity, ranging from 0.985 to 0.999. In sum, there is not much allozyme variation in this closely related, highly inbred sample of the Benjamin Constant population.

Correlations between allozyme heterozygosity and morphological traits and growth parameters were generally not significant and did not show any trend that might suggest heterosis. Since most of the phenotypic variation of the traits listed in Table 7.6 has a large environmental component (see Chapter 6), and the very low heterozygosities strongly suggest a recent history of inbreeding, the absence of correlations that would suggest heterosis is not surprising. This small pejibaye sample is composed of closely

related, highly inbred germplasm, in what is considered an allogamous species. Thus, the lack of heterotic affects is an additional confirmation of the high degree of inbreeding in the recent history of this germplasm.

Although no trends were observed between allozyme heterozygosity and growth, allozyme analysis will be useful in a future breeding program to fingerprint the outstanding individuals in the population and guide a hybridization plan. Since heterozygosity is one of the probable explanations for heterosis (Simmonds 1979, Mitton 1989), allozyme analysis can be used to identify genetically divergent populations for hybridization to maximize heterosis. It can be used on the resulting progeny to identify those plants that are the result of hybridization, since there is a high likelihood of selfing under controlled pollination in pejibaye because of the difficulty of emasculating the staminate flowers.

8.7. A Pejibaye Ideotype for Hawai'i

Since Donald (1968) introduced the term *ideotype*, breeders have adopted it as a framework for the objectives of their breeding programs. As Simmonds (1979) points out, however, the more elaborate the ideotype the slower the progress to reach it. Thus, an ideotype is most useful if it contains a few major traits.

Pejibaye has been selected by humans for thousands of years (Clement 1988, 1992). Amerindian ideotypes, however, were quite different from what the Hawaiian farmer and consumer will want. Nonetheless, their selections serve as the basis for future advances.

In Hawai'i, the most important traits are:

1) **Spineless petioles**. Farmer-owners will demand spineless petioles because it makes management much easier. Perhaps as important, it will reduce their insurance liability in the event that a farm laborer is injured by spines.

Spineless germplasm is available from the Benjamin Constant (Brazil), Yurimaguas (Peru) and San Carlos (Costa Rica) populations already introduced into Hawai'i. Further introductions from all populations are essential, especially from Costa Rica because of the small number of spineless plants that resulted from the first introduction. Heritability is apparently low, but response should be immediate and complete, because only spineless germplasm will be used as parents. Care must be used in characterizing spininess in mature plants with seed production potential, in order to avoid mis-characterization caused by the developmental shift from spiny to spineless found in this study.

2) **Fast growth** (= high RGR). Although farmers will need to stagger their deliveries to restaurants, all palms must be fast growers. Environmental variation can be counted on to create enough variation in harvest date in a plantation to guarantee continual yield throughout the year. Nursery growth can be a preliminary indicator of fast growth, supplemented by earliness of first harvest.

Several Benjamin Constant progenies contain large numbers of fast growing plants, especially progenies B-9, B-0, B-1 and B-2. Several Yurimaguas progenies appear to be even faster growing, but require further analysis. Cloning, via tissue culture, can facilitate the identification and immediate exploitation of the fastest growing individual plants in Hawai'i. This is unlikely to be a good long-term solution in tropical America, however,

because of co-evolved pests that could expand their populations dramatically on very uniform materials. Heritability is moderate, but response may be slow because of the true amount of variability available for this selection.

3) **Continual offshoot production**. Offshoots guarantee the next harvest cycle, so all plants must produce a reasonable number of offshoots over all harvest cycles. The germplasm selected for hybridization or cloning must be evaluated over several cycles to confirm its offshoot production. This aspect of selecting germplasm will take longer than any other because it requires data from several harvest cycles.

The majority of the plants in the Benjamin Constant and Yurimaguas introductions produce sufficient offshoots. A moderate number of offshoots produced early in shoot development is preferable to a large number, because excess offshoots must be pruned to allow adequate space in the plantation for each stem. Heritability is apparently low, but there is enough within progeny variation to permit a reasonable response.

4) **Low acridity**. Since all palms contain calcium oxalate crystals, the identification of other components of acridity (probably an enzyme) is essential to be able to evaluate plants for this quality trait. Contrary to the observation of Tabora et al. (1993) that some consumers like the 'bite' of a palm heart, this negative trait could slow acceptance of fresh palm heart and even leave restaurants and farmers open to possible liability suits if an extremely allergic person suffered medical complications from eating the product.

There appears to be variability for acridity in the germplasm available in Hawai'i. Nothing is known about its amount, distribution, or interaction with environments, however, because the only way to evaluate it at present is by tasting each palm. Since

acridity-sensitive people do not often volunteer for this type of torture, a complete evaluation awaits the development of a better assay for acridity.

Individual palm yield has not been included in the above list for two reasons: (1) there is relatively little variation present in these progenies (although the Yurimaguas progenies consistently yield somewhat more than the Benjamin Constant progenies) and heritability estimates are relatively low; and (2) fast growth is more important at this stage than individual palm yield because a fast growing palm will produce a larger number of hearts over time than a moderate or slow grower.

This is a much shorter ideotype than that presented by Clement et al. (1988) and should give a much more rapid response, especially if coupled with clonal propagation. To get a rapid response, however, more germplasm will be necessary and it will need to be adequately evaluated with growth analysis methods.

Appendix A: Descriptions of the Soils Used in this Study.

A.1. **Hilo Series** (Sato et al. 1973). The Hilo series is an Andisol: Typic Hydrudand, hydrous, isohypothermic.

The Hilo series consists of well-drained silty clay loams. These soils formed in a series of volcanic ash layers that give them a banded appearance. They are gently sloping to steep soils on uplands at an elevation ranging from near sea level to 800 feet. They receive from 120 to 180 inches of rainfall annually, and their mean annual soil temperature is between 72° and 74° F. These soils are in the same general area as Kaiwiki, Olaa, and Ookala soils.

Hilo silty clay loam, 0 to 10 percent slopes. - This soil is low on the windward side of Mauna Kea and is dissected by deep, narrow gulches.

In a representative profile the surface layer is dark-brown silty clay loam about 12 inches thick. The subsoil is about 48 inches thick and consists of dark-brown, dark reddish-brown, and very dark grayish-brown silty clay loam. The surface layer is very strongly acid, and the subsoil is strongly acid to medium acid. This soil dehydrates irreversibly into fine gravel-size aggregates.

Representative profile, Honomu Quadrangle, latitude 19°46'50" N and longitude 155°05'45" W.

Ap - 0 to 12 inches, dark-brown (10YR 3/3) silty clay loam; strong, fine, subangular blocky structure; friable, slightly sticky, and plastic; many roots; many

medium and fine pores; very strongly acid; abrupt, smooth boundary. (10 to 13 inches thick)

B21 - 12 to 22 inches, dark-brown (7.5YR 3/2) silty clay loam; moderate, medium, prismatic structure that breaks to moderate, medium and fine, subangular blocky structure; hard, friable, slightly sticky, plastic, and moderately smeary; many roots; many fine and very fine pores; many, fine, sugar-like coatings; strongly acid; clear, smooth boundary. (10 to 12 inches thick)

B22 - 22 to 31 inches, dark reddish-brown (5YR 3/4) silty clay loam; moderate, medium, prismatic structure that breaks to strong, medium and fine, subangular blocky structure; extremely hard, friable, slightly sticky, plastic, and moderately smeary; many roots; many medium and fine pores; many, fine, sugar-like coatings; medium acid; abrupt, smooth boundary. (7 to 10 inches thick)

B23 - 31 to 34 inches, very dark grayish-brown (10YR 3/2) silty clay loam; strong, medium, prismatic structure that breaks to strong, medium, subangular blocky structure; extremely hard, firm, slightly sticky, plastic, and moderately smeary; common roots; many medium and fine pores; few hard ash nodules; medium acid; abrupt, smooth boundary. (2 to 3 inches thick)

B24 - 34 to 42 inches, dark-brown (7.5YR 3/4) and dark reddish-brown (5YR 3/4) silty clay loam; moderate, medium, prismatic structure that breaks to a strong, medium and fine, subangular blocky structure; extremely hard, friable,

slightly sticky, plastic, and strongly smeary; few roots; many medium and fine pores; medium acid; abrupt, smooth boundary. (8 to 11 inches thick)

B25 - 42 to 46 inches, dark-brown (7.5YR 3/4) silty clay loam; moderate, medium, prismatic structure that breaks to strong, fine, subangular blocky structure; extremely hard, friable, sticky, plastic, and smeary; few roots; many coarse to very fine pores; medium acid; abrupt, smooth boundary. (4 to 11 inches thick)

B26 - 46 to 60 inches, dark-brown (7.5YR 3/4) silty clay loam; moderate, fine, prismatic structure that breaks to strong, fine, subangular blocky structure; extremely hard, friable, sticky, plastic and smeary; few roots; many fine and medium pores; medium acid.

The depth to bedrock is more than 5 feet. The B horizon, when moist, ranges from 5YR to 10YR in hue and from 2 to 4 in chroma.

Included in mapping are small areas of shallow soils over pahoehoe lava bedrock.

Permeability is rapid, runoff is slow, and the erosion hazard is slight. Roots can penetrate to a depth of 5 feet or more.

Hilo silty clay loam, 10 to 20 percent slopes. - This soil is similar to Hilo silty clay loam, 0 to 10 percent slopes, but steeper. Runoff is medium, and the erosion hazard is slight to moderate.

Hilo silty clay loam, 20 to 35 percent slopes. - This soil is similar to the others, but is steeper. Runoff is medium and the erosion hazard is moderate.

Two other descriptions of Hilo series soils, with full mineralogical and chemical analysis, are provided by the Soil Conservation Service (1976).

A.2. **Keaukaha Series** (Sato et al. 1973). The Keaukaha series is an Histosol: Lithic Tropofolist, dysic, isohypothermic.

The Keaukaha series consists of well-drained, thin organic soils overlying pahoehoe lava bedrock. The soils occupy the low areas of Mauna Loa. The are at an elevation ranging from near sea level to 1,000 feet and receive from 90 inches to more than 150 inches of rainfall annually. Their mean annual soil temperature is between 72° and 74°F.

Representative profile, Hilo Quadrangle, latitude 19°39'05" N and longitude 155°03'46" W.

O2 - 8 inches to 0, very dark brown (10YR 2/2) muck; moderate, fine, subangular blocky structure; friable, slightly sticky, slightly plastic, and moderately smeary; many roots; many fine pores; strongly acid; abrupt, wavy boundary. (3 to 10 inches thick)

IIR - 0 to 10 inches, pahoehoe lava.

The depth to pahoehoe lava bedrock ranges from 3 to 10 inches. The hue of the O2 horizon ranges from 7.5YR to 10YR.

The soil above the lava is rapidly permeable. The pahoehoe lava is very slowly permeable, but water moves rapidly through the cracks. Runoff is medium, and the erosion hazard is slight. In places, roots are matted over the pahoehoe lava or extend a few feet into the cracks.

A.3. **Wahiawa Series** (Foote et al. 1972). The Wahiawa series is an Oxisol: Rhodic Eutrustox, clayey, kaolinitic, isohyperthermic.

This series consists of well-drained soils on uplands on the island of Oahu. These soils developed in residuum and old alluvium derived from basic igneous rock. They are nearly level to moderately steep. Elevations range from 500 to 1,200 feet. Rainfall amounts to 40 to 60 inches annually; most of it occurs between November and April. The mean annual soil temperature is 71°F.

Wahiawa silty clay, 0 to 3 percent slopes. - This soil occurs on smooth, broad interfluves.

In a representative profile the surface layer is very dusky red and dusky red silty clay about 12 inches thick. The subsoil, about 48 inches thick, is dark reddish-brown silty clay that has subangular blocky structure. The underlying material is weathered basic igneous roch. The soil is medium acid in the surface layer and medium acid to neutral in the subsoil.

Permeability is moderately rapid. Runoff is slow, and the erosion hazard is no more than slight. The available water capacity is aobut 1.3 inches per foot in the surface layer and about 1.4 inches per foot in the subsoil. In places roots penetrate to a depth of 5 feet or more.

Representative profile: Island of Oahu, latitude 21°26'16" N and longitude 158°00'16" W.

Ap1 - 0 to 6 inches, very dusky red (2.5Y 2/2) silty clay, dusky red (2.5YR 3/2) when dry; moderate, medium, fine and v ery fine, granular structure; very hard, friable, sticky and plastic; abundant roots; many medium, fine and very fine, interstitial pores; many black concretions 0.125 inch to 0.25 inch in diameter;

violent effervescence with hydrogen peroxide; medium acid; abrupt, smooth boundary, 2 to 6 inches thick.

Ap2 - 6 to 12 inches, dusky-red (2.5YR 3/2), moist and ry, silty clay; commonly, dark reddish-brown (2.5YR 3/4) material from the B horizon of cultivated soil; moderate, coarse, subangular blocky structure; hard, firm, sticky and plastic; abundant roots; few, fine and very fine, tubular pores; compact in place; many black concretions; violent effervescence with hydrogen peroxide; medium acid; abrupt, wavy boundary. 5 to 8 inches thick.

B21 - 12 to 16 inches, dark reddish-brown (2.5YR 2/4) silty clay, dark reddish brown (2.5YR 3/4) when dry; moderate, fine and very fine, subangular blocky structure; hard, firm, sticky and plastic; plentiful roots; common, fine and very fine, tubular pores and few, coarse, tubular pores; many black concretions; strong effervescence with hydrogen peroxide; medium acid; gradual, wavy boundary. 4 to 8 inches thick.

B22 - 16 to 33 inches, dark reddish-brown (2.5YR 2/4) silty clay, dark reddish brown (2.5YR 3/4) when dry; moderate and strong, fine and very fine, subangular blocky structure; hard, friable, sticky and plastic; few roots; common, fine and very fine, tubular pores; nearly continuous pressure cutans; many, fine, distinct, black stains; few black concretions; stong effervescence with hydrogen peroxide; slightly acid; diffuse, wavy boundary. 14 to 20 inches thick.

B23 - 33 to 45 inches, dark reddish-brown (2.5YR 2/4) silty clay, dark reddish brown (2.5YR 3/4) when dry; moderate and strong, very fine, subangular blocky structure; hard, friable, sticky and plastic; common, fine and very fine, tubular pores; nearly continuous pressure cutans; many, fine, distinct, black stains; few black concretions; moderate effervescence with hydrogen peroxide; neutral; diffuse, wavy boundary. 10 to 14 inches thick.

B24 - 45 to 60 inches, dark reddish-brown (2.5YR 2/4) silty clay, dark reddish brown (2.5YR 3/4) when dry; moderate and strong, very fine, subangular blocky structure; hard, friable, sticky and plastic; common, fine and very fine, tubular pores; few, fine, black stains; thin, patchy clay films; continuous pressure cutans; many distinct slickensides as much as 2 inches long; very few black concretions; slight effervescence with hydrogen peroxide; neutral.

Black concretions, 2 to 5 mm in size, occur on the surface and to a depth of 5 feet or more. The depth to highly weathered basalt ranges from 5 feet to more than 10 feet. A few boulder cores occur in the lower part of the solum in places. The A horizon ranges from 2 to 3 in value and from 2 to 4 in chroma when dry or moist. The B horizon ranges from 2.5YR to 10YR in hue; from 2 to 3 in value when dry or moist; and from 3 to 6 in chroma when dry and from 3 to 5 in chroma when moist.

Wahiawa silty clay, 3 to 8 percent slopes. - On this soil, runoff is low and the erosion hazard is slight. Included in mapping were small areas of nearly level soil.

Wahiawa silty clay, 8 to 15 percent slopes. - On this soil, runoff is medium and the erosion hazard is moderate. Included in mapping were small areas that are stony and eroded.

Wahiawa silty clay, 15 to 25 percent slopes, eroded. - Most of the surface layer of this soil, and in places part of the subsoil, has been removed by erosion. The profile is otherwise like that of Wahiawa silty clay, 0 to 3 percent slopes. The depth to soft weathered rock ranges from 2 to 3 feet. Boulders occur on the surface in a few places. Runoff is medium to rapid, and the erosion hazard is severe. Included in mapping were small stony areas.

Appendix B: Summary of Data Used for Allometry Study

B.1. The Bifid Eophyll (section 4.5.1.)

Table B.1.1. Descriptive statistics of the 28 pejibaye bifid eophylls used to validate previous allometric equations and to explore new ones.

	rachis				blade			leaf	leaf
	length (cm)	width (mm)	thick (mm)	w x t (mm^2)	length (cm)	width (cm)	l x w (cm^2)	area (cm^2)	wt (g)
mean	18	3.4	4.1	15.1	38.3	8.5	374.1	414.7	4.62
SD	9.2	1.1	1.1	8.2	13.9	3.68	275	316.4	4.01
CV	51.1	32.4	26.8	54.3	36.3	43.3	73.5	76.3	86.8
min	3.5	1.9	2.1	4.2	16	2.5	40	50.9	0.51
max	37	6.1	6.9	40	69	16.0	1035	1264.9	15.84

Table B.1.2. Correlation matrix of the pejibaye bifid eophyll dimensions in Table B.1.1.

	rachis				blade			leaf
	length	width	thick	w x t	length	width	l x w	area
r. wide	0.866							
r. thick	0.824	0.749						
r. w x t	0.901	0.944	0.893					
b. long	0.971	0.894	0.819	0.913				
b. wide	0.934	0.952	0.782	0.919	0.948			
b. l x w	0.964	0.933	0.791	0.945	0.971	0.968		
leaf area	0.963	0.925	0.800	0.954	0.966	0.947	0.995	
leaf wt	0.896	0.932	0.747	0.943	0.900	0.904	0.955	0.964

The critical value of r is 0.5974, for p = 0.001.

B.2. The Pinnate Leaf Area

Table B.2.1. Descriptive statistics of the 25 pinnate pejibaye leaves used to validate previous allometric equations and to explore new ones.

	rachis				leaflet		leaf
	length (m)	width (mm)	thickness (mm)	number	length (m)	width (m)	area (m^2)
mean	1.13	16.1	14.6	119.7	0.47	0.025	0.835
SD	0.49	5.56	4.08	48.65	0.11	0.004	0.469
CV	43.4	34.5	27.9	40.7	23.4	16.0	56.1
min	0.38	6	7	41	0.28	9.014	0.082
max	2.16	25	21	204	0.65	0.032	1.657

Table B.2.2. Correlation matrix of the pinnate pejibaye leaves in Table B.2.1.

	rachis				leaflet		rect.
	length	width	thick	#	length	width	area
r. width	0.877						
r. thick	0.903	0.971					
leaflet #	0.965	0.881	0.903				
l. length	0.728	0.811	0.794	0.665			
l. width	0.494	0.638	0.714	0.508	0.741		
rec area	0.938	0.945	0.945	0.925	0.874	0.688	
leafarea	0.971	0.920	0.931	0.940	0.824	0.626	0.979

Critical values of r are: 0.4227, 0.5368, and 0.6524, for p = 0.05, 0.01 and 0.001, respectively.

B.3. The Pinnate Leaf Biomass

Table B.3.1. Descriptive statistics of the 20 pinnate pejibaye leaves used to validate previous leaf biomass allometric equations and to explore new ones.

	petiole	rachis				leaf	weights			
	length	length	width	thick	w * t	area	petiole	rachis	leaflet	leaf
	(m)	(m)	(mm)	(mm)	(cm^2)	(m^2)	(g)	(g)	(g)	(g)
mean	0.78	1.29	19.2	16.5	3.35	1.021	71.5	25.5	88.7	185.6
SD	0.14	0.37	5.14	3.65	1.46	0.384	34.7	12.3	39.0	85.0
CV	17.9	28.7	26.8	22.1	43.6	37.3	48.5	48.4	44.0	45.8
min	0.52	0.53	10	10	1.00	0.289	16.8	4.3	21.1	44.0
max	0.94	1.83	26	21	5.46	1.469	130.1	40.9	138.3	298.7

Table B.3.2. Correlation matrix of pejibaye pinnate leaf dimensions in Table B.3.1.

	petiole	rachis				leaf	weights		
	length	length	width	thick	w*t	area	petiole	rachis	leaflets
r long	0.829								
r wide	0.831	0.863							
r thick	0.826	0.831	0.982						
r w*t	0.799	0.815	0.988	0.989					
lf area	0.877	0.946	0.961	0.944	0.932				
p wt	0.860	0.738	0.938	0.964	0.952	0.891			
r wt	0.860	0.867	0.983	0.974	0.972	0.960	0.936		
lflet wt	0.853	0.857	0.985	0.984	0.985	0.963	0.963	0.983	
leaf wt	0.867	0.820	0.977	0.986	0.981	0.945	0.985	0.978	0.994

The critical value of r is 0.6787, for p = 0.001.

B.4. The Whole Plant

Table B.4.1. Descriptive statistics for height (ht), leaf number (lvs), whole plant leaf area (lfar) and whole plant biomass (plwt) of the 27 pejibaye plants used to generate allometric equations. All plants (n = 27). Bifid eophylls plants (n = 9). Pinnate leaved plants (n = 18).

	All Plants				Bifid Eophyll				Pinnate Leaved			
	ht (m)	lvs	lfar (m^2)	plw (kg)	ht (m)	lvs	lfar (m^2)	plw (kg)	ht (m)	lvs	lfar (m^2)	plw (kg)
mea	0.61	6.33	3.09	0.71	0.15	5.44	0.18	0.02	0.84	6.78	4.55	1.06
SD	0.48	1.25	3.20	0.84	0.06	0.83	0.11	0.02	0.43	1.18	2.99	0.84
CV	79	20	103	118	40	15	63	71	51	17	66	80
min	0.08	4	0.037	0.004	0.08	4	0.037	0.004	0.29	5	0.69	0.10
max	1.7	9	10.01	2.90	0.23	7	0.38	0.06	1.7	9	10.01	2.90

Table B.4.2. Correlation matrix of plant dimensions in Table B.4.1.

	All Plants			Bifid Eophylls			Pinnate Leaved		
	ht	lvs	lfarea	ht	lvs	lfarea	ht	lvs	lfarea
# leaves	0.517			-0.094			0.313		
leaf area	0.977	0.547		0.906	0.196		0.963	0.373	
biomass	0.980	0.525	0.984	0.925	0.173	0.984	0.983	0.369	0.981

Critical values of r are: A. 0.3809, 0.4869, 0.5974, for p = 0.05, 0.01, 0.001, respectively, for n = 27; B. 0.6664, 0.7977, 0.8982, for n = 9; C. 0.4683, 0.5897, 0.7084, for n = 18.

Appendix C: Density and Position Effects

Minitab's (1991) GLM routine was used to determine the effect of position within plot and the effect of density on leaf number and plant height at harvest at Ninole. A split-split-plot model was used for this test. Only the effects of density and position on leaf number and plant height are tested here (see F_c values). All other single-plant growth measurements and parameters presented similar results.

C.1. Analysis of Variance for Number of Leaves at Harvest

Source	DF	Seq SS	Adj SS	Adj MS	F_f	P	F_c
block	2	13.680	11.464	5.732	5.01	0.007	
density	2	7.062	5.236	2.618	2.29	0.103	0.73ns
blck*dens	4	15.460	14.277	3.569	3.12	0.015	
position	2	0.893	0.598	0.299	0.26	0.770	0.37ns
pos*dens	4	2.615	1.759	0.440	0.38	0.820	0.55ns
pos*blck	4	3.523	3.659	0.915	0.80	0.526	
pos*dens*blck	8	6.107	6.808	0.851	0.74	0.653	
Prog	6	96.489	95.050	15.842	13.84	0.000	
Prog*dens	12	23.705	23.580	1.965	1.72	0.061	
Prog*pos	12	17.928	16.634	1.386	1.21	0.273	
Prog*blck	12	10.533	11.060	0.922	0.80	0.645	
Prog*pos*pos	24	19.062	19.062	0.794	0.69	0.859	
Error	399	456.863	456.863	1.145			
Total	491	673.919					

C.2. Analysis of Variance for Plant Height at Harvest

Source	DF	Seq SS	Adj SS	Adj MS	F_f	P	F_c
block	2	0.47097	0.35461	0.17731	9.83	0.000	
density	2	0.01458	0.01351	0.00676	0.37	0.688	0.78ns
blck*dens	4	0.05269	0.03446	0.00862	0.48	0.752	
position	2	0.03755	0.02263	0.01132	0.63	0.535	0.67ns
pos*dens	4	0.00614	0.01040	0.00260	0.14	0.966	0.15ns
pos*blck	4	0.05255	0.04448	0.01112	0.62	0.651	
pos*dens*blck	8	0.15128	0.12221	0.01528	0.85	0.562	
Prog	6	0.41087	0.41205	0.06868	3.81	0.001	
Prog*dens	12	0.42557	0.37442	0.03120	1.73	0.058	
Prog*pos	12	0.11753	0.15558	0.01296	0.72	0.733	
Prog*blck	12	0.35755	0.35932	0.02994	1.66	0.073	
Prog*dens*pos	24	0.51115	0.51115	0.02130	1.18	0.255	
Error	399	7.19808	7.19808	0.01804			
Total	491	9.80651					

Appendix D: Mean Progeny Product Weights

Table D.1. Adjusted mean±SD potential heart-of-palm, edible stem, edible leaf, and total edible weight per plant (g) up to the end of the first harvest for each pejibaye progeny at Ninole.

Progeny	Heart	Stem	Leaf	Total
B-0	183±56	393±92	59±31	635±125
B-1	174±55	377±80	52±28	603±121
B-2	165±52	402±85	63±28	630±128
B-3	185±51	398±110	62±35	644±150
B-5	191±63	398±108	54±28	643±158
B-8	177±62	409±111	59±30	645±150
B-9	180±46	428±92	53±29	662±127
mean	178±55	401±98	58±30	637±137

[a] assuming 5000 plants/ha and all plants harvested

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